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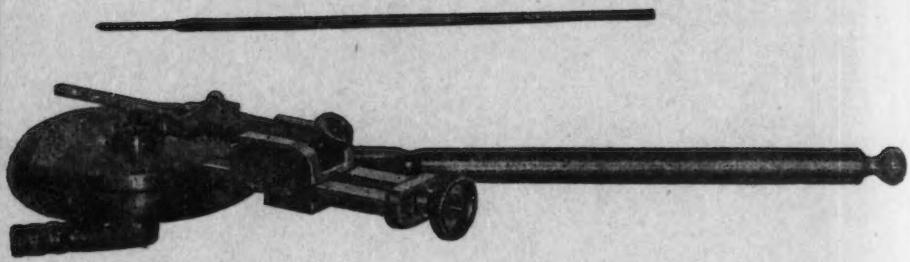

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PHYSIOLOGICAL VARIATIONS IN THE CARDIAC OUTPUT OF MAN

XII. The Effect of the Menstrual Cycle on the Cardiac Output, Pulse Rate, Blood Pressure, and Oxygen Consumption of a Normal Woman

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Received for publication August 16, 1930

The cyclic nature of the menstrual function in woman has led observers to seek a relationship between the changes in various physiological functions and the catamenial cycle. In a previous paper of this series (Grollman, 1930), determinations carried out on a male subject over the course of a year and a half were reported. It was shown that the cardiac output and its related functions were constant (within the limits of the experimental errors) from day to day throughout the year, if precautions were taken to maintain strictly basal conditions. Simultaneously with the above experiments, a series of determinations was made on a female subject. The results, although showing marked constancy, did not preclude the possibility that the menstrual cycle might be reflected in the daily variations of the cardiac output. The determinations on the female subject were, therefore, continued daily for a period of several menstrual cycles. Every precaution was taken to avoid all factors other than the truly physiological effects of the catamenial cycle on the cardio-vascular and metabolic systems. The present paper gives the results of this investigation.

Methods. In the study of physiological functions such as pulse rate, oxygen consumption, cardiac output, *etc.*, which are easily affected by extraneous factors, it is essential that all variables other than the one studied be most stringently controlled. This is particularly true in a study of the effects of menstruation. Any pain or discomfort (physical or psychical) can produce changes which might be erroneously attributed to the catamenial cycle when, in reality, they are only secondary effects. In order to avoid the possibility of any such factors influencing the present

results and to obtain as strictly basal conditions as possible, the present determinations were made immediately after the subject's spontaneous awakening in the morning after a normal night's sleep. The pulse rate was counted and the blood pressure determined graphically before the subject had made any physical movements after awakening. The cardiac output was then determined, as previously described (Grollman, 1930). After a resting period of about five minutes, the oxygen consumption was determined with the Krogh spirometer which was placed beside the subject's bed. A second cardiac output was then determined which concluded the experiment. All of the above mentioned procedures were carried out in about fifteen minutes and did not necessitate the subject's movement from bed. These precautions are much more rigid than those which previous authors have usually followed and the constancy of the results recorded in this paper may be attributed to the maintenance of such conditions. The same precautions as regards details of the procedure, —*e.g.*, the insistance of a good night's rest on the part of the subject, a comfortable room temperature, *etc.*, were the same as those described in the previous study of the male subject (Grollman, 1930).

The subject of the present study was a healthy, nulliparous, married woman, age 28. Her menses were regular, occurring at 26 day intervals, with normal flow of 4 days' duration, and were completely devoid of pain, discomfort, or any interference with her daily work.

Results. Since the general constancy and seasonal independence of the various factors studied as obtained on the female subject of the present investigation were essentially the same as those previously reported for a male subject (Grollman, 1930) the results obtained over the course of several years will not be given. The present paper will limit itself to a consideration of the results obtained from day to day throughout the menstrual cycle to determine the effect, if any, of this cycle on the observed changes in the various functions studied.

In table 1 is given a series of typical results covering one complete cycle. The most striking fact which is noted in this table is the high degree of constancy which the various factors show throughout the experimental period. Thus the pulse rate, which is notorious for its variability, varied only between 48 and 55, with an average of 52. The blood pressure is remarkably constant. The oxygen consumption shows a degree of variation which previous observers have also noted. The individual values vary from 180 to 214 with an average of 199. The extreme variations range, therefore, from −9.5 per cent to +7.5 per cent of the average value. The cardiac output shows a remarkable constancy, varying from 3.15 to 3.47 liters, with an average of 3.30 liters. There is thus a maximum deviation in the individual values of only 5 per cent from the mean which is the magnitude of the unavoidable experimental errors of the method utilized.

Considerable work has been done by previous investigators to determine if the catamenial cycle is reflected in cyclic changes in other physiological functions. To determine the bearing of the present findings on the existence of such rhythmic variations, figure 1 has been constructed which gives a curve of the determinations of the pulse rate and oxygen consumption during a single cycle and data of the cardiac output for two cycles.

TABLE 1

The daily variations of the pulse rate, blood pressure, oxygen consumption, and cardiac output of a normal, female subject during the course of a menstrual cycle

RELATION OF DATE TO MENSTRUAL CYCLE	PULSE RATE	BLOOD PRESSURE	OXYGEN CONSUMPTION	CARDIAC OUTPUT
	per minute	*mm. of Hg*	*cc. per minute*	*liters per minute*
1st day of menstruation	50	102/71	208	3.35
2nd day of menstruation	53	96/64	208	3.30
3rd day of menstruation	53	102/67	198	3.30
4th day of menstruation	51	105/71	198	3.30
1st day after cessation of menses	48	98/65	212	3.31
2nd day after cessation of menses	50	100/66	197	3.28
3rd day after cessation of menses	48	101/66	210	3.44
4th day after cessation of menses	52	100/64	189	3.15
5th day after cessation of menses	55	98/64	214	3.45
6th day after cessation of menses	52	97/65	193	3.22
7th day after cessation of menses	52	97/67	214	3.45
8th day after cessation of menses	52	101/68	204	3.34
9th day after cessation of menses	54	100/65	189	3.15
10th day after cessation of menses	53	100/66	190	3.17
11th day after cessation of menses	50	100/65	196	3.16
12th day after cessation of menses	49	100/65	199	3.26
13th day after cessation of menses	51	101/66	212	3.21
14th day after cessation of menses	54	101/66	202	3.37
15th day after cessation of menses	55	102/66	196	3.16
16th day after cessation of menses	54	103/70	180	3.38
17th day after cessation of menses	53	98/65	204	3.20
18th day after cessation of menses	53	105/70	189	3.32
19th day after cessation of menses	50	94/60	201	3.47
20th day after cessation of menses	53	95/57	190	3.33
Day preceding onset of menses	53	102/71	190	3.33
Average	52	100/66	199	3.30

The daily blood pressure changes are obviously so small (as shown in table 1) as to preclude the necessity of further comment.

Pulse rate. The liability of the pulse rate makes any attempt at its correlation with the menstrual cycle exceedingly difficult. The mere acts of dressing and coming to the laboratory (as has been the procedure in most previous investigations) suffice to affect this function and to

preclude any hopes of obtaining regular changes in it. The procedure used in the present study, however, involving as it did measurement immediately on awakening, should permit the attainment of data which would show any regularity in the pulse rate changes that might occur.

As seen in figure 1, the pulse rate changes regularly from day today giving a remarkably uniform curve. With the free use of imagination one might even ascribe to the curve a definite relationship to the menstrual cycle. Thus well defined maxima occur in the curve 7 days before the onset of the menses and 5 days after its cessation. Minima occur during menstruation and 10 days after its cessation. Such an interpretation, which indeed has been often made on the basis of less well-defined curves by previous observers in regard to the changes of various functions with the catamenial cycle, is, however, not justifiable. It is merely cited to indicate the ease with which daily variations of a given function may be made to

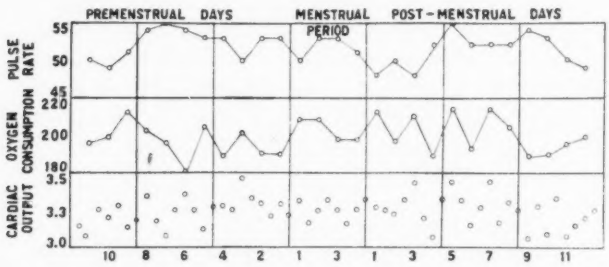

Fig. 1. The effect of the menstrual cycle on the pulse rate, oxygen consumption and cardiac output. The *abscissae* indicate days; the *ordinates* indicate the values of the various functions investigated.

appear as following some particular rule. The fact that the changes between the various maxima and minima occur with fairly good regularity is to be sought in the fact that the factors causing them develop gradually.

Oxygen consumption. Previous studies on the effect of the menstrual cycle on the basal metabolism are in marked disagreement as shown in the review of Du Bois (1927). Zunz (1906), Blunt and Dye (1921), Wiltshire (1921), and others have found no consistent variations: Snell, Ford, and Rowntree (1920), Hafkesbring and Collett (1924), Collett and Liljestrand (1924), Benedict and Finn (1928), Griffith, *et alii* (1929), and others report variations which they claim are related to the menstrual cycle. Wakeham (1923) demonstrated the large individual variations occurring in a group of healthy subjects so that the conclusions of many of the above mentioned authors being based as they often are on one subject are not convincing. From the available evidence and discrepancy in the findings of

different workers it must be concluded that although the basal metabolism of women may vary about ±8 per cent from its mean value, no exact relationship necessarily obtains between this variation and the menstrual cycle. Indeed, the various workers who have reported a definite relationship between menstruation and metabolism by no means agree as to the nature of this relationship. Thus some record maximum values during menstruation (*e.g.*, Snell, Ford, and Rowntree, 1920), others minimum (*e.g.*, Benedict and Finn, 1928) while others claim the existence of several definite maxima and minima (*e.g.*, Collett and Liljestrand, 1924).

The results of the present study, as exemplified in the curve of figure 1, show no definite obvious relationship between the basal metabolism and the catamenial cycle.

Cardiac output. A previous attempt to correlate cardiac output changes with the menstrual cycle has also been made by Collett and Liljestrand (1924). These observers concluded as a result of their study on one subject over several catamenial cycles that the cardiac output reached a maximum 7 days after menstruation, a minimum several days later, underwent a secondary premenstrual rise, and reached a second minimum on the first day of the menstrual period. Although their observed variations were irregular they considered a periodic tendency to be clear. The values of the cardiac outputs observed by Collett and Liljestrand varied, however, from 2.6 to 5.0 liters. Such a large individual variation has not been observed in any of the subjects ever studied by the author. Collett and Liljestrand's results are, therefore, attributed to experimental errors due to incomplete mixing. The failure to attain homogeneous mixture, as the chief vitiating factor in determinations of the cardiac output, has repeatedly been referred to in the present series of papers on cardiac output. Individuals have been encountered in whom the attainment of such mixture is extremely difficult. The conclusions reached by Collett and Liljestrand from other experiments on the same subject as was used in the study of the menstrual cycle have also not been confirmed (Grollman, 1929a). This fact adds to the probability of the above explanation being the cause of the discrepancies.

The results of determinations during two menstrual cycles, which are plotted in figure 1, clearly show the haphazard distribution of the experimental values. As pointed out above, the total variation from the mean (table 1) is only 5 per cent which is the absolute limit of accuracy of the method utilized. We must thus conclude that on the subject studied during the present investigation any menstrual variation of the cardiac output must be less than 5 per cent, and is not detectable by present methods of investigation. The constancy of the blood pressure values and the changes in pulse rate which were observed also speak against the existence of any detectable cardio-vascular relation to the catamenial cycle.

DISCUSSION. It should be emphasized here that the subject of the present experiments suffered no malaise, pains, or other distressing disturbances at menstruation. There was present a very vague, but in no wise disturbing, feeling of abdominal fullness just before the onset of the menses but even this premonition was usually absent. It is quite possible that in subjects in whom the menstrual cycle is associated with a train of mental and physical symptoms, secondary changes in the cardiac output may be expected (Grollman, 1929c).

The conclusions of the present paper are based on experiments on a single individual, the nature of the work having made application to a greater number of subjects impossible. However, during the course of the experiments cited in previous papers of this series, particularly in the study of the normal value of the cardiac output (Grollman, 1929b) a number of female subjects have been studied. In none of these have values differing widely from the normal predicted value been obtained despite the fact that the determinations were made without reference to the stage of the catamenial cycle. This would speak against the existence of any wide menstrual variation of the cardiac output of normal women.

It must be admitted that the curves for the pulse rate and basal metabolism as given in table 1 show a regular rhythmic periodicity which might be considered as related to the menstrual cycle. A similar periodicity whose existence is masked by the experimental errors of the methods employed, might also be attributed to the cardiac output changes. However, it seems unjustifiable to consider the curves of table 1 (or the similar curves which previous authors have published) as entirely and directly due to menstrual influences. If the observed curves were truly reflections of changes in the organism due to some menstrual variation, one would expect them to be more uniform. One would not expect maximal values to occur in one woman where minimal values occur in another. Nor would one expect a minimum pulse rate (as occurs on the 6th premenstrual day of table 1) to be accompanied by a maximum basal metabolism. In view of the conflicting conclusions based on non-convincing data which the numerous previous observers have found we must conclude that any truly menstrual variation of these functions is of such small magnitude as to be over-shadowed by the much greater daily variations which occur due to other causes. Moreover, where definite maximum or minimum values are found to occur at some period of the menstrual cycle, such changes are equally well attributable to factors which are merely secondary to this cycle. In the author's opinion, the available evidence justifies the conclusion that *the menstrual cycle produces only slight changes in the various physiological functions of normal women and that we must attribute the more marked changes observed in certain cases as results of secondary factors complicated by experimental errors of observation.* It must be noted, however,

that the present conclusions are based on observations on a single individual. It is quite possible that some women might show periodic variations in the cardiac output during the menstrual cycle, as has been claimed for other physiological functions.

SUMMARY

A study was made of the variations of the pulse rate, blood pressure, oxygen consumption, and cardiac output in relation to the catamenial cycle. No definite menstrual variations in these functions were observed despite the extra precautions taken to attain perfect basal conditions and the avoidance of all known vitiating factors. The pulse rate varied from 48 to 55 during the cycle and showed periodic and regular changes whose significance, however, was minimized. The blood pressure was extremely constant throughout the cycle. The oxygen consumption varied from 180 to 214 cubic centimeters per minute but no definite relationship between these variations and the menstrual cycle could be demonstrated. The cardiac output showed a constancy from day to day within 5 per cent of the mean value, which variation was well within the predictable experimental error. It was concluded that variations in the metabolic and cardiovascular functions bear no simple rhythmic relationship to the catamenial cycle as has often been maintained.

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PHYSIOLOGICAL VARIATIONS IN THE CARDIAC OUTPUT OF MAN

XIII. The Effect of Mild Muscular Exercise on the Cardiac Output

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A knowledge of the changes in cardiac output which accompany muscular exercise is essential for an understanding of this important physiological state. Unfortunately the methods available at present for determining the cardiac output render these determinations difficult under severe exercise and fraught with considerable error even in comparatively mild exercise. The previous extensive investigations on the subject by Krogh and Lindhard and subsequent workers using their method (Krogh and Lindhard, 1912; Boothby, 1915; Newburgh and Means, 1915; Lindhard, 1915, 1920, 1923; Liljestrand and Lindhard, 1920; Liljestrand and Stenström, 1922; Collett and Liljestrand, 1924; Jarish and Liljestrand, 1927) were confined almost entirely to moderate and severe exercise. In many physiological problems, *e.g.*, postural changes, exposure to cold, forced breathing, *etc.*, it is necessary to know what effect the muscular activities, which accompany such changes, produce in the cardiac output. Results obtained during the investigations described in the previous papers of this series have led to the belief that many of the current views regarding the effect of light exercise on the cardiac output were incorrect. The present investigation was, therefore, undertaken to determine the effect of very mild exertion (such as involves an increase in oxygen consumption of only several hundred cubic centimeters per minute) on the cardiac output.

Methods. The same procedure was used for the determination of the cardiac output as in the preceding papers of this series (Marshall and Grollman, 1928; Grollman, 1929 a). Unfortunately this method does not permit the accurate determination of the cardiac output when the latter is greatly increased and it was therefore necessary to limit the determinations to very mild exercise. The failure to attain homogeneous mixture during the rebreathing procedure is most liable to lead to error in determining the cardiac output by the above method. One must however, also, avoid exceeding the time of a single circulation of the blood. In the procedure of Krogh and Lindhard (1912) this last consideration was minimized by

taking "half samples" of alveolar air, and hence their method is applicable to more violent exercise, where the circulation is greatly accelerated. This advantage is, however, counterbalanced by the difficulty and uncertainty of the "mixing" which introduces great errors.

The subject upon whom the data recorded in the present paper were obtained, could mix thoroughly, as determined by experiments with hydrogen (Grollman and Marshall, 1928), in 10 to 12 seconds. The analytical accuracy with which acetylene can be determined makes it possible to avoid any appreciable error in experiments during exercise when the period between the collection of the two samples is only about 3 seconds. Hence it was possible in this subject to complete a determination in 13 to 15 seconds. Assuming the decrease in circulation time of the blood to be proportional to the increase in cardiac output, one may obtain values almost twice as great as the normal without sacrifice of accuracy. When the cardiac output is greater than 7 liters, however, the errors involved in overpassing the time of a single circulation render the accuracy of the results questionable. During moderate or severe exercise (when the cardiac output is over 10 liters) the results are quite undependable even on the above mentioned subject whose ability to mix was unusually good. For this reason, the results quoted in the present paper are limited to values of the cardiac output which are not greater than 7.0 liters and, in this range, are considered to be practically as accurate as those obtained in the resting condition.[1]

[1] Since completing the work described in the present paper, Baumann and the author (1930) have demonstrated the dependability and accuracy of the cardiac output determinations when performed under the conditions defined above. We have, in the first place, compared the results obtained by the use of acetylene with those derived from a determination of the cardiac output by direct analysis of the blood obtained from the human right heart. The latter procedure has been limited heretofore to experiments on the lower animals but the technique of heart puncture makes the method also applicable to man. By comparing the oxygen content of the blood removed from the right human ventricle with that obtained from the brachial artery one obtains an unequivocal and direct measure of the cardiac output. The average deviation of a series of determinations simultaneously carried out by this direct method from those obtained by the use of acetylene was only 2 per cent. We have also investigated two other fundamental principles upon which the indirect method using acetylene is based. Simultaneous determinations of the tension of acetylene in the alveolar air with that in the blood demonstrated that, in normal individuals at least, acetylene distributes itself in accord with the physico-chemical laws of the solution of gases in liquids. The same distribution of the gas was found to occur *in vivo* (with the attainment of a true equilibrium) as had previously been demonstrated to occur *in vitro*. The question of the circulation time in man is also fundamental to an understanding of the validity of cardiac output determinations. All previous attempts to answer this question have been either indirect or have given at best only qualitative results. We have been able to solve the problem quantitatively and directly by determining the acetylene content of blood removed from the

All the experiments quoted were done in the basal condition and with the precautions noted in the preceding papers of this series.

RESULTS. It is a widely current notion that the cardiac output is a simple function of the oxygen consumption during exercise (Boothby, 1915, Krogh and Lindhard, 1917), although exceptions to this view have been previously noted (Lindhard, 1915). Thus Starling's textbook (1930) cites the curves of Means and Newburgh (1915) in which the cardiac output is plotted against the oxygen consumption during work. Although this concept is, in a sense, true within certain limits of oxygen consumption, in which the same muscle groups are predominantly active, it leads to quite erroneous conceptions, particularly in considering very mild exercise. That the cardiac output is not determined solely by the oxygen consumption and may vary over wide limits for the same expenditure of energy (as measured by the oxygen consumption) is demonstrated in table 1. This table gives the results of 3 sets of experiments involving various types of muscular activity. In comparing experiment 5 with experiments 2 and 3, one notes that the cardiac output in the former (when the forearms are alternately flexed once a second) is less than in the latter (when only one arm is flexed once per second as in experiment 2, or even twice per second as in experiment 3). The oxygen consumption in experiment 5 is only slightly greater than that in experiment 2 and less that that of experiment 4. Nevertheless the cardiac output is much less than it is in either experiments 2 or 3. Although the work expended in flexing the arms alternately once a second is about the same as that in flexing one arm every second and less than that expended when both arms are flexed once per second, the heart output is less in the first case.

Experiments 7 and 8 of the second series of table 1 also demonstrate the independence of the cardiac output to the oxygen consumption or work expended. Thus, alternately flexing both thighs results in a much smaller cardiac output than does the flexion of one thigh every second, although the work and oxygen consumption in the two cases are the same.

Experiments 10 and 11 of the third series of table 1 show the comparatively high cardiac outputs which may be obtained with small oxygen expenditures. Thus the flexion and extension of the fingers of both hands, although requiring only 100 cubic centimeters of oxygen over the basal value, results in a marked increase in the cardiac output as does also a

right human heart at varying intervals after beginning to rebreathe acetylene as in the procedure for determining the cardiac output. In normal resting subjects the amount of acetylene returning to the lungs at the end of the experimental procedure (20 to 23 seconds after beginning the rebreathing) is negligible. When the cardiac output is increased, however, the circulation time is proportionately diminished. The above described experiments offer direct and unequivocal evidence for the accuracy of the cardiac output determinations described in the papers of this series.

corresponding muscular movement of the feet. The cardiac output is much greater in either case than for the same oxygen consumption as observed in the muscular movements of the first 2 series of table 1. In experiments 12 and 13, on the other hand, the increases in cardiac output are much less than those of experiments 10 and 11 despite the greater oxygen consumption in the former experiments. Experiments 12 and 13 represent a type of static exercise as compared to the dynamic exercise of

TABLE 1

The relation of the cardiac output to the oxygen consumption in mild exercise

EXPERIMENT NUMBER	TYPE OF EXERCISE	OXYGEN CONSUMPTION	ARTERIO-VENOUS OXYGEN DIFFERENCE	CARDIAC OUTPUT
		cc. per minute	*cc. per liter*	*liters per minute*
1	Resting	246	60	4.1
2	Flexing and extending right forearm, once per second	286	59	4.8
3	Flexing and extending right forearm, twice per second	340	65	5.2
4	Flexing and extending right forearm rapidly	453	76	6.0
5	Alternately flexing and extending both forearms, each every other second	315	73	4.3
6	Resting	256	61	4.2
7	Flexing right thigh once per second	430	56	7.7
8	Alternately flexing both thighs, each every other second	428	85	5.0
9	Resting	240	60	4.0
10	Flexing and extending hand muscles, twice per second	346	55	6.3
11	Flexing and extending feet and toes, once per second	310	62	5.0
12	Supporting body in horizontal position with feet flexed	424	72	5.9
13	Supporting body in horizontal position with feet flexed	352	66	5.3

experiments 10 and 11. We must thus conclude that the cardiac output and the cardio-vascular response to muscular activity, in general, are independent of the oxygen consumption when different groups of muscles are involved.

The question arises as to the effect of increasing the activity of a given muscular group on the cardiac output and its related functions. As may be noted in the experiments of table 1, the arterio-venous oxygen difference for very slow muscular movements (*e.g.*, experiment 5) is high and the

cardiac output consequently is but slightly raised. With increasing activity the arterio-venous oxygen difference falls (as in experiment 2) after which it again rises (experiments 3 and 4). The cardiac output gradually increases with greater muscular activity at a progressively diminishing but not uniform rate. Very quick movements (as in experiments 10 and 11) result in rapid increases in the cardiac output.

The effect on the cardiac output of gradual increases in the activity of the muscle groups involved in walking is demonstrated in table 2. When the subject is standing still, he is using the muscles which maintain the upright posture, but these are comparatively static. The arterio-venous oxygen difference under these conditions is high and the cardiac output is the same as in the sitting position (Grollman, 1928). Any decrease in

TABLE 2

The relation of the cardiac output to the oxygen consumption with varying degrees of muscular exertion of essentially the same muscle groups

CONDITION OF EXPERIMENT	OXYGEN CONSUMPTION	ARTERIO-VENOUS OXYGEN DIFFERENCE	CARDIAC OUTPUT
	cc. per minute	*cc. per liter*	*liters per minute*
Leaning against a wall (rested)	289	80	3.6
Standing quietly (rested)	339	85	4.0
Sliding feet once every 1½ seconds	347	83	4.2
Standing quietly after work	374	86	4.4
Walking 1 step every 5 seconds	403	79	5.1
Walking 1 step per second	500	87	5.8
Walking 1 step per second	527	89	5.9
Walking 1 step per second	538	92	5.9
Walking 1 step per second	550	89	6.2
Walking 1 step per second	605	95	6.4
Raising feet, once per second	607	101	6.0
Raising feet, twice per second	728	104	7.0

cardiac output due to the effect of gravity is counteracted by a compensatory cardio-vascular reaction. With a further increase in the oxygen consumption (as in walking) the arterio-venous oxygen difference also increases but not proportionately to the former. Hence there is a gradual rise in the cardiac output with greater muscular activity.

DISCUSSION. The cardio-vascular reactions to muscular exercise consist of a complex series of changes occurring in various parts of the organism. Prior to and during work there may be widespread reaction due to stimuli from the higher centers. This includes an increase in blood pressure, pulse rate, and, very likely, in cardiac output. The mild forms of exercise cited in tables 1 and 2 required little effort for their performance (with the exception of experiments 12 and 13 of table 1) and hence central stimulation of the cardiac output in these cases is improbable.

Other important changes during muscular activity are a dilatation and opening of capillaries and an increased venous return due to the pumping action of the muscles. The last two named factors may be considered as the prime agents in increasing the blood flow in mild exercise. The blood pressure rise which occurs under these conditions is so slight as to be a minor factor in producing the observed increases in cardiac output. The heart may thus be considered as merely reacting to the increased venous return, as discussed by Eyster (1926). According to this view, the difference in response of the cadiac output to the various forms of exercise of table 1 would be explicable as due to a variation in the intensity with which the muscular activity aided in the return of blood to the heart. Exercises involving efficient venous emptying (as in experiment 10 of table 1) would result in a much greater cardiac output than a static exercise (as in experiment 12). Such muscular activity as is involved in standing or shivering in response to cold would result, as has been previously demonstrated. (Grollman, 1928, 1930 c) in slight increases in cardiac output because they do not involve active aid in returning blood to the heart. Rapid movements, on the other hand, produce marked changes in cardiac output. The increases in cardiac output observed during forced breathing (Grollman, 1930 b) may thus, in large part at least, be merely resultants of the increased blood flow through the active muscles of respiration.

The above view stresses the importance of the muscles as active aids in maintaining the circulation in exercise. The discrepancy in the results of Chauveau and Kaufmann (1887) and of Tschuewsky (1903) are explicable on this basis. The former noted a 4- to 8-fold increase in the blood flow through the *levator propius labii superioris* of the horse during activity while the latter noted much slighter increases when the *gracilis* of the dog was stimulated. In sustained tetanic contraction, as might be predicted, Tschuewsky found even a smaller response in cardiac output.

Lindhard (1915, 1920) has also demonstrated the dependence of the rate at which work is performed on the cardiac output. Thus for a given expenditure of energy, the cardiac output during swimming was greater than it was while riding on a bicycle ergometer. This difference is explained by Kisch (1927) as due to the effect of cold in the swimming experiments. This explanation seems improbable from the results previously described (Grollman, 1930 c). It is more probable that the muscular movements vary in their effectiveness in returning the venous blood to the heart and thus produce the observed changes. The effect of training on reducing the response of the cardiac output, as found by Lindhard (1920) and by Collett and Liljestrand (1924), may also, in part at least, be explicable on the same basis. The better coördination of movements which results from training leads to an economy of movement and a lesser return of blood to the heart for a given amount of work.

In static work, Lindhard (1920) has also observed a greater increase in cardiac output than would be expected from the oxygen utilized. This result is surprising in view of the fact that one would expect a slight response, as indeed Lindhard himself anticipated, due to the lack of muscular movements in such static work. However, in the experiment of Lindhard (hanging on a horizontal beam), there is considerable nervous stimulation due to the strain of the exercise. Under such conditions the nervous factors controlling the cardio-vascular system probably help to produce the observed increases. That such purely nervous influences do occur is evidenced by the cadio-vascular response preparatory to exertion or to psychic stimulation in general (Grollman, 1929 b). This psychic factor was recognized by early workers (Moritz, 1903; Masing, 1902). Thus Masing showed that work performed with a single leg caused a greater blood pressure rise than when the same amount of work was done by the two legs alternately. Moritz, indeed, considered the pressure elevation during bodily work to be principally conditioned by psychical influences.

During an investigation of the effect of high altitudes on the cardio-vascular system (Grollman, 1930 a), experiments were performed similar to those of table 2 in order to compare the reaction of the cardiac output to exercise under low barometric pressure with that at sea-level. The results obtained before acclimitization was established (about 10 days) were higher than those obtained for the same exercise at sea level. However, if correction was made for the observed increased basal values during this period, it was found that the increased cardiac output due to the exercise was equal to that observed in the same experiment at sea-level. Experiments performed when the subject had become acclimatized (and when the basal resting cardiac output had resumed its sea-level) showed the same increases in cardiac output as were observed at sea-level.

SUMMARY

A study was made of the cardiac output during very mild muscular activity. The cardiac output in such exercise bears no simple relation to the oxygen consumption. Active muscular movements result in a much greater increase of the cardiac output than static or slow movements. This is explained as due to the greater pumping action of the muscles in the former exercises which causes a greater return of venous blood to the heart.

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STUDIES OF SINGLE MUSCLE FIBRES

III. Further Evidence of Graded Responses in Single Fibres[1]

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That single muscle fibres are capable of submaximal contractions, and that graded unit responses can be elicited by changes in stimulus intensity has already been shown by the writer (1930) for the musculature of the retrolingual membrane (membrana basihyoidea) of the frog. Pratt (1930), to a limited extent, was also able to observe these unit partial contractions in the same musculature, although his records made from this tissue *in situ* show discontinuous, all-or-none type of responses. He has since, however, more completely, confirmed the author's results by the use of a variety of microelectrodes.[3]

In attempting to account for the failure of the all-or-none law the author (1930) called attention to the method that permitted localization of the stimulus to a single fibre, and the delicate grading of the stimulus intensity. Since the experiments were performed on the musculature of the retrolingual membrane only, the possibility that graded responses were characteristics of this type of vertebrate striated muscle only, was left open. For the experiments reported in this paper the sartorius muscle of the frog was used.

All the experiments were carried out on the curarized sartorius *in situ*, with intact circulation. The same method of microstimulation and recording (Pratt and Eisenberger recording method) that was described in the previous paper was here used. The whole animal is placed in a glass chamber that is mounted on the mechanical stage of a compound microscope. The thigh muscles are exposed and sprayed with minute mercury droplets. The chamber is then filled with frog's Ringer solution sufficiently to keep the muscle submerged. The records are photographs of the excursions of minute mercury globules as produced by the contracting fibres.

Figure 1a and b is a continuous record of the responses of a single fibre,

[1] Abstract of paper, as given at Chicago meeting of American Physiological Society, March 1930, in this Journal, xciii, 650.

[2] Donnelley Fellow in Physiology.

[3] Report at Chicago meeting of American Physiological Society, March, 1930, abstracted in this Journal, xciii, 680.

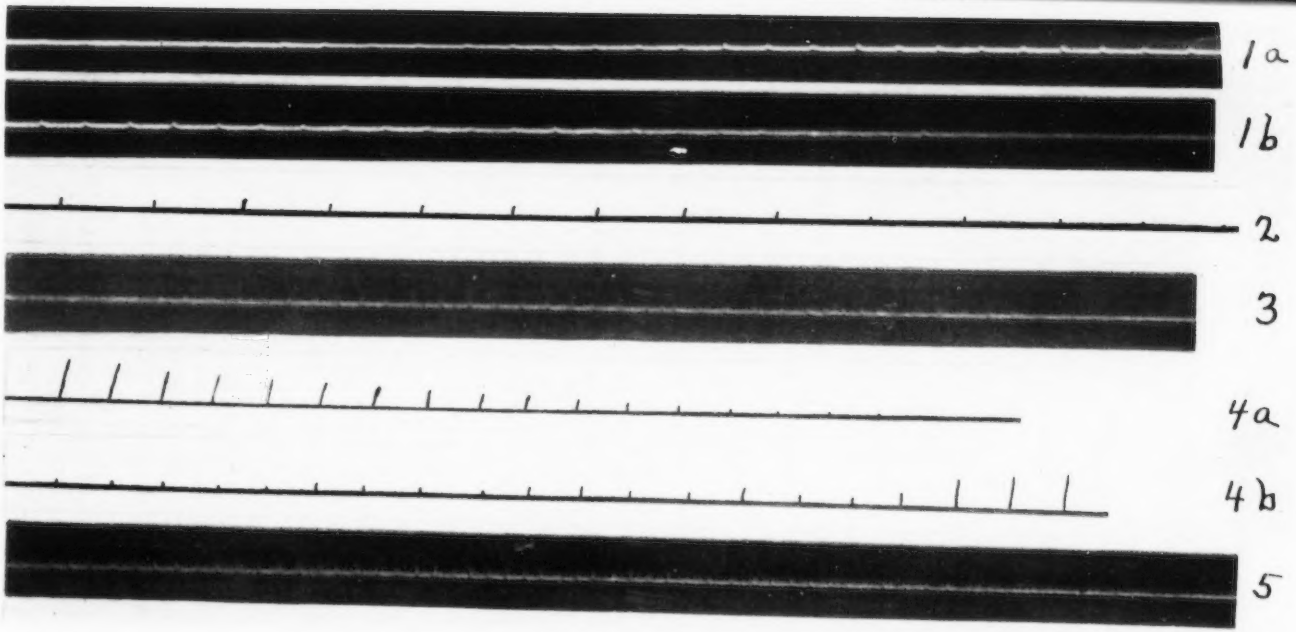

Fig. 1a and b. Continuous record of the responses of a single fibre of the curarized sartorious (R. pipiens) *in situ* and with circulation intact. Total magnification ×100. Two microelectrodes of nicrome wire 26μ in diameter, encased in quartz, used. Break induction shocks every 2 seconds delivered by metronome interrupter. Stimuli graded by movement of secondary coil and rheostat in circuit. Record reads from left to right.

Fig. 2. Tracing of the projected image of negative film of first half of figure 1a. Total magnification ×200. Record reads from right to left.

Fig. 3. Smaller responses are those of a single fibre, larger ones represent additional units. As additional units are excited, both the graded and discontinuous steps are to be seen. Total magnification ×100. Break induction shocks 40 per minute. Other conditions same as in figure 1. Record reads from left to right.

Fig. 4a and b. Tracing of the projected image of negative of record of figure 3. Total magnification ×200. Record reads from right to left.

Fig. 5. Effect of using larger electrodes, platinum drawn out in quartz to 50μ in diameter. Total magnification ×80. Break induction shocks 40 per minute. Record reads from right to left. A distinct tendency to the discontinuous and step-like gradations.

showing graded contractions to graded strengths of stimuli. As a rule, when the stimulus intensity becomes great enough to excite additional fibres, the all-or-none effect is produced. Figure 3, and its enlarged tracing in figure 4, indicates both the continuous and the discontinuous gradations. The smaller responses (compare with fig. 1) represent a single fibre, and the larger responses indicate the introductions of additional contracting units.

With microelectrodes the stimulus can easily be localized to a single fibre. To produce submaximal contractions in a single and intact fibre, not only microelectrodes must be used, but they must not exceed a certain size. In figure 5 the distinct and predominant tendency to the discontinuous and all-or-none response can be seen. In this experiment platinum electrodes 50 μ in diameter were used for stimulation. With the excellent microscopic visibility that is possible in the study of the musculature of the retrolingual membrane, the effect of increasing the size of the electrode can most clearly and convincingly be observed. Using the nonpolarizable Ag, AgCl, agar microelectrodes of about 2 to 5 μ in diameter, the submaximal responses can always be evoked in the intact single fibre. Using metal, encased in quartz, microelectrodes of about 25 μ in diameter, the graded response becomes more difficult to elicit in normal and intact fibres. With still further increase in the size of the electrode it becomes still more difficult and finally impossible to produce submaximal responses. In the previous paper (Gelfan, 1930) the importance of intimacy of contact of electrodes and fibre has already been pointed out.

Brown and Sichel (1930) in a very recent paper have demonstrated that the myograms from a single fibre of the frog's sartorius, isolated by excision, show a progressive increase in the magnitude of each response with increasing strengths of stimuli. Friedheim (in press) also, has observed single skeletal fibres, naturally isolated in a tissue culture preparation, to give graded responses to changing strengths of stimuli.

Hartree and Hill (1921) found that the ratio of heat produced to tension developed, H/T, does not remain constant with increasing strengths of stimuli, as is to be expected according to the all-or-none law, but increases. They conclude that the character of the response of the single fibre, as well as the number of fibres responding, alters as the strength of the stimulus is increased. They consider, therefore, that within limits at any rate, the all-or-none law to fail. Fischer (1930) also, claims an absence of all-or-none in the heat production of skeletal muscle after direct stimulation.

According to Fenn (1923), "The energy liberated by the contraction of a single muscle fibre for a given stimulus is not dependent solely upon the initial mechanical and physiological condition of the muscle but can be modified by the nature of the load which the muscle discovers it must lift

after the stimulus is over." This effect has been confirmed by Hartree and Hill (1928) for tetanic contractions but not for the single twitch.

If the all-or-none conception were now to be limited only to the statement that the response of the muscle fibre is independent of the strength of the stimulus, it would no longer hold. With proper microstimulation the responses of a fresh and uninjured single muscle fibre can be graded by graded strengths of stimuli. We must, therefore, conclude that the contractile mechanism of a skeletal fibre is of such a nature as to permit graded responses, and that it is not independent of the stimulus intensity. In this respect, the all-or-none law for muscle is no longer valid. The previous work on muscle with direct stimulation, in which the law was found to hold, must be attributed to diffuse stimulation. With indirect stimulation, if the all-or-none law is valid for nerve, as most of the evidence indicates to be the case,[4] only maximal responses would be produced since the nervous impulse discharged to the muscle fibre is always all-or-nothing.

After extirpation of the nerve cord, Carlson (1905) found that the magnitude of the responses of the Limulus heart, upon direct stimulation, varied, within limits, directly with the strength of the stimulus. With the nerve cord intact, however, there is a greater tendency to uniform contractions, when stimulated by varying intensities. From the number of invertebrate hearts that Carlson (1906) investigated he concluded that with one or two exceptions, they showed the tendency to the all-or-none type of response, but these same hearts when in poor condition will give graded responses. Similarly, Kronecker and Mays (1883) showed that the frog's heart when fatigued or otherwise in poor condition exhibits graded contractions in response to graded intensities of stimulation.

If we were to consider, as Gelfan and Gerard (1930) have done, the conductile mechanism as distinct, to some degree at any rate, from the contractile one, the difference in behavior between the fresh heart and the one in poor condition might be explained in the following manner. Although the contractile mechanism permits submaximal responses, the transmissive mechanism may do so to a much less extent. It is conceivable, therefore, that the latter mechanism may be impaired in tissues that are stale, injured, or otherwise in poor condition, and that the graded responses are due to the inoperation of the conductile mechanism. An all-or-none response may then be said to be obtained by virture of the operation of the transmissive mechanism. This, of course, assumes that contraction may take place independently of the excitatory process. That the "products of the excitation reaction are not necessary for the

[4] Failure of the all-or-none law has recently been reported for vertebrate nerve by Mansfield, Hecht and Kovacs (1929) and Lanczos (1929), and by Jordan (1928) for invertebrate nerve.

actual process of contraction," is claimed by Burridge (1928). He (1929) further holds that the all-or-none law in heart is a characteristic of the excitation process as distinct from the contractile one.

It is interesting to point out that Hintner (1930) could obtain submaximal responses in the retrolingual membrane by diffuse stimulation if he cut the fibres. The intact fibres, on the other hand, with diffuse stimulation, he found to give only maximal responses, in contrast to what Fischl and Kahn (1928) found. The graded responses in the intact fibres of the retrolingual membrane and the sartorius that are obtained by microstimulation, may therefore be due, as Gelfan and Gerard have advanced, to a direct stimulation of the contractile mechanism, made possible by extreme localization of stimulus without, to a limited extent, involving the transmissive one. The other possible explanation is decremental conduction.

SUMMARY

In the intact frog's sartorius, graded contractions of single fibres are obtained in response to graded intensities of stimuli.

To evoke submaximal responses in a single fibre that is fresh and uninjured, the microelectrodes must not exceed a certain size, so that the localized stimulation of the single fibre is not too diffuse.

Since the response is not independent of the stimulus intensity, upon direct microstimulation, the all-or-none law for the muscle, to that extent, is not valid.

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THE EFFECT OF INTRAVENOUS AND INTRAPERITONEAL INJECTIONS OF IRRADIATED ERGOSTEROL[1]

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This investigation was begun in the hope that administration of ergosterol by some route other than by mouth would throw some light on the mechanism of action of this very potent agent. While the original purpose has not fully materialized some of our observations seem to be significant. The literature is quite confusing but the more recent work indicates that irradiated ergosterol given by mouth is not toxic except in doses that are hundreds or even thousands of times as large as the therapeutic dose. (Kreitmair and Moll, 1928; Schoenholz, 1929; Harris and Stewart, 1929; Harris and Moore, 1928; Klein, 1929; Pfanenstiel, 1928; Light, Miller and Frey, 1929; Bills and Wirick, 1930; Shohl and Brown, 1930; Hess, Weinstock and Rivkin, 1929; Hottinger, 1929; Holtz and Von Brand, 1929.)

Several investigators have found clinical evidence of toxic effects in infants receiving large doses. (Wurzinger, 1928; György, 1929; Hess and Lewis, 1928.) Still others have failed to produce toxic effects by overdosage (Nomura, 1929; Cornel, 1929; Borghi, 1929; Dixon and Hoyle, 1928; Hoyle and Buckland, 1929; Cartland, Speer and Heyl, 1929). Hoyle (1930) has confirmed the earlier reports that toxicity occurs with ergosterol irradiated in alcoholic solution and in addition has made the important observation that toxicity is related to the diet.

Heubner (1929) and Harris and Moore (1929) believe that toxicity and therapeutic potency are parallel and due to the same principle although this point is still open to question. Our experiments do not confirm this conclusion.

To the best of our knowledge the work of Munehisa (1929) and of Von Brand and Holtz (1929) represents the only recorded attempts to administer ergosterol by routes other than by mouth. However, Laurens (1928) cites several experiments on the administration of cod liver oil by intraperitoneal, subcutaneous or intramuscular injection or by direct cutaneous application.

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It is the purpose of this paper to show that this substance is more effective when given by other routes than by mouth, or is effective in smaller quantities. Dogs were selected as subjects because of the necessity for securing large samples of blood at frequent intervals. Normal animals were used because of our desire to try to localize the physiological mechanism affected by the substance and it was felt that this could be done more readily on normal than on rachitic animals.

The intravenous administration of ergosterol has the advantage of insuring a constant application of a given dosage since it is probable that some ergosterol is reduced to coprosterol in the intestinal tract. This action may vary quantitatively at different times. It seems, therefore, that alimentary administration does not insure a constant application to the effective mechanism. Two preparations have been used, irradiated ergosterol supplied by the Standard Products Company—10 mgm. per cubic

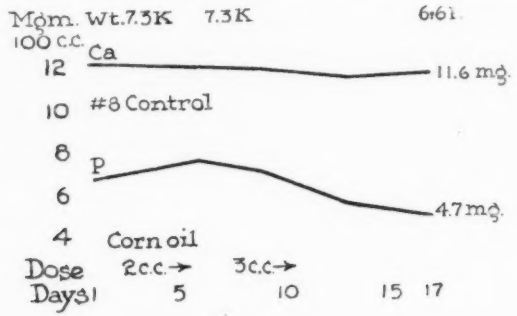

Fig. 1

centimeter of corn oil—and Viosterol (superacterol) supplied by Mead, Johnson and Company. This also contained 10 mgm. per cubic centimeter. Two different lots of the first and three of the latter have been employed. In our experiments comparable doses showed no differences in effectiveness. All dogs were confined to a stock diet of ground beef heart and puppy meal, 3:1. Routine determinations of calcium and inorganic phosphorus were made on heparinized heart blood. Preliminary observations were made on all animals. Calcium concentration was determined by the method of Clark and Collip (1925), inorganic phosphorus by that of Fiske and Subbarow (1925).

Experimental results. Since the procedure employed involved the intravenous injection of relatively large amounts of corn oil it was deemed necessary to first determine the effects of this substance on control animals. In figure 1 are shown graphs of calcemia and phosphemia from one of these animals. In our experience the alterations in inorganic phosphorus are

within the normal range for dogs in confinement. The most significant thing is the absence of fluctuations in calcemia so evident in the other animals. This case is typical of all the controls. In one instance 15 cc. of corn oil were injected at one time into the saphenous vein without any discoverable reaction. Microscopic examination of this animal's lungs on the following day revealed no emboli. Injections were made slowly through a fine needle so that the oil evidently emulsified readily. Colleagues in this institution report injection of still larger quantities of inert oils without injury. Our experience confirms that of Nomura (1929).

Dog 1. Initial weight 10 K. On the 5th day of confinement, 5 mgm. of ergosterol were injected. On the 9th day the dose was increased to 20 mgm. and continued to the 20th day. There was considerable fluctuation in the calcium concentration but the general average level increased progressively for 10 days. It is noteworthy that the initial increase was induced by 0.5 mgm. per kilo of body weight. Up to the end of the 15th day the animal gained weight in spite of a tendency to greater activity than is usually displayed by dogs under similar conditions. This increased activity was displayed by several animals of this series and is apparently a manifestation of beneficial effects. The animal had received a total of 240 mgm. of ergosterol up to the 20th day when it was found that calcemia had returned to the initial level and the daily dose was increased to 30 mgm. for 8 days, making a total of another 240 mgm. On the 28th day the daily dose was increased to 50 mgm. for 3 days so that the animal received a grand total of 630 mgm., or on the basis of initial weight, 63 mgm. per kilo. Hypercalcemia appeared immediately and continued progressively to the 31st day when death occurred.

The highest figure was 18.7 mgm. per 100 cc. From the 20th day there was a progressive loss to the terminal weight of 6.2 K. Along with this manifestation there was progressive loss of appetite, cachexia, sluggishness, extreme emaciation. Neither diarrhea nor vomiting were noted. Microscopic examination of tissues revealed extensive calcium deposits in the kidneys and general degenerative changes in the tubular epithelium.

The inorganic phosphorus fluctuations are of little significance as they are within the normal range.

It seems probable that the dog developed some tolerance to the smaller dosage as employed during the beneficial period, since calcemia rose and fell again. Also, it is possible that this reaction accounts for the failure of some investigators to find hypercalcemia after long continued administration of an optimal dosage.

In an effort to determine whether the toxicity apparent in dog 1 was related to the hypercalcemia and also whether it was related to the size of dosage or to total administration, dogs 2 and 3 were given larger amounts over short periods. Both of these animals developed all the toxic symp-

toms described but neither showed any pronounced hypercalcemia. Death occurred on the 4th and 5th days respectively. From these and other similar cases in our series, it is apparent that toxicity and hypercalcemia are not closely interrelated.

Dog 9. Intraperitoneal injection. Weight remained constant until the 31st day after which there was rapid loss during the next 7 days. There was no pronounced hypercalcemia until after the 28th day although a total of 910 mgm. had been administered up to this time. On the basis of initial weight this would mean approximately 125 mgm. per kilo. After this time, up to the 32nd day, 250 mgm. more were injected, making a grand total of 1160 mgm. During the last 6 days no ergosterol was administered. Nevertheless calcemia increased progressively to 16.5 mgm. After the 20th day it was possible to withdraw oil from the peritoneal cavity and at death on the 38th day about 10 cc. were recovered for spectroscopic examination which was made by Dr. C. E. Bills. Potency was only $\frac{1}{10}$ that of the original oil. Whether this decreased potency was due to dilution or to selective absorption of ergosterol from the oil could not be determined with the small amount available.

Toxic symptoms began to appear about the 20th day and became progressively more pronounced although the weight loss was delayed. Apparently the threshold of susceptibility of this animal was rather high. Also there was evidently slow absorption of the ergosterol from the peritoneal reservoir.

Various tissues were taken for both microscopic and chemical examination.

These and similar experiences suggested another line of procedure. Dog 5 (fig. 2). Young adult dog, well nourished, initial weight 10.7 K. Preliminary observations extended over 6 days. During the next 8 days 300 mgm. (approximately 29 mgm. per kilo) of ergosterol were injected intravenously, in doses as shown in detail in the chart. Two days after the last injection calcemia had increased from 10.2 to 12 mgm., and two days later to 13.75. Thus there was a rather long latent period for the appearance of hypercalcemia. No toxic symptoms were manifest and the dog gained weight rapidly and continued at 12.2 K. until about the 40th day.

After the 17th day no further observations were made until the 35th day when calcemia was 11.7 mgm. It is probable that a higher figure than 13.75, as observed on the 17th day, had been reached in the interim. On the 38th day injections were resumed. Whereas the initial dosage of 20 mgm. daily had produced no response there was now an immediate response and a hypercalcemia of 15 mgm. had developed on the 45th day. This increase had begun to appear after a total of 60 mgm. had been administered, as against 200 mgm. in the initial period, before hypercalcemia appeared. On the 45th day the dose was increased to 30 mgm. daily until

a total of 350 mgm. had been injected in the second period. In the two periods, with an interval of 25 days, the dog received a grand total of 650 mgm. of ergosterol. Since there was still some hypercalcemia when the second treatment period was begun it seems probable that there was still a considerable store of ergosterol in the body. This would account for the more prompt response to the second period of treatment although it does not preclude the possibility that the animal may have become sensitized by the first treatment.

The injections were finally discontinued after the 51st day, but the increase in calcium continued progressively until the 71st day when the high figure of 22.2 mgm. was attained. On the 74th day the calcium concentration had fallen to 15.16 mgm. when inorganic phosphorus had increased from 4.4 mgm. on the 63rd day to 9.58 mgm. The animal died later in the day. The reason for these terminal changes is not apparent at present.

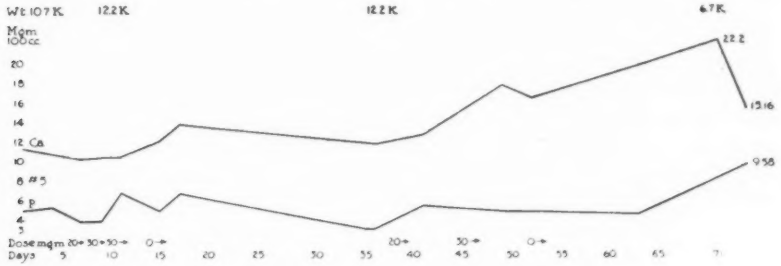

Fig. 2

After the 40th day the dog lost weight rapidly, and developed the typical toxic symptoms described above.

In all, our series comprises 14 dogs to which irradiated ergosterol has been administered by one of the methods described. The detailed descriptions given are typical of our experience with the others.

Microscopic examination of tissues has revealed calcium deposits only in the kidneys. The only evidence of tissue injury was found in a rather uniform degeneration of the kidney tubule epithelium.

Chemical analyses of tissues for content of calcium, phosphorus and potassium are not yet completed to such an extent as to justify inclusion but will be reported later.

Since it is apparent that administration of ergosterol by routes other than the alimentary tract gives more nearly quantitative responses, it is suggested that this method be utilized in further study of the pharmacology of this substance. It is even possible that this method of administration might be found of value in certain clinical cases.

CONCLUSIONS

1. Irradiated ergosterol administered by intravenous or intraperitoneal injection is more effective than comparable doses by mouth. The alimentary tract protects to some extent against the toxic effects of large doses.

2. Present available evidence indicates that the induction of toxic symptoms is not necessarily parallel with hypercalcemia.

3. Present evidence does not indicate any constant effect of ergosterol when administered by this method on the concentration of inorganic phosphorus in the blood.

4. Continued administration of a therapeutic dose of ergosterol may develop in the normal animal a certain degree of tolerance as indicated by the reduction of hypercalcemia.

5. Hypercalcemia may persist for long periods after administration has been discontinued.

6. After pronounced toxic symptoms were developed, none of the animals in this series recovered after discontinuance of administration.

Thanks are due Mead, Johnson & Co. and Standard Products Co. for generous supplies of irradiated ergosterol; to Mr. R. I. Klein for the tissue analysis; to the staff of the Department of Pathology for the microscopic work and to Dr. C. E. Bills for valuable suggestions, and to the various subsidies that have supplemented the financial resources available for the work.

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BIOCHEMICAL STUDIES ON THE EFFECT OF ADRENALIN UPON THE NITROGEN METABOLISM OF RABBITS

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In connection with the biochemical studies on the effect of corpus luteum we have previously reported (1) that the administration of adrenalin to rabbits results in blood sugar and blood urea rises similar to those following the intraperitoneal injection of corpus luteum into rabbits that are in the reactive state. In the paper referred to it was shown that doe rabbits at times reacted to corpus luteum, given intraperitoneally, with typical blood sugar and blood urea rises, whereas at other times no blood changes resulted. The reaction could be predicted with certainty 1, in normal doe rabbits on the second day after being served by a buck and for a variable number of days thereafter; 2, in lactating does on the day after delivery and for 8 to 12 days after; 3, in bucks and in spayed does that had previously been given folliculin. Table 1 demonstrates the similarity of the corpus luteum and adrenalin reactions. It has been shown that in both instances the total rise in non-protein nitrogen of the blood is accounted for by rise in urea.

Although the principal interest of the writers has been the study of the mechanism of the action of corpus luteum, this finding of an apparent effect of adrenalin upon nitrogen metabolism seemed sufficiently important to warrant further investigation. The possible significance of the similarity between the corpus luteum and adrenalin reactions was discussed in our last communication. The present report will confine itself to some further biochemical studies on the reaction of rabbits to adrenalin.

In this work the same Folin micro methods for blood analysis have been followed as in the corpus luteum investigations. The Folin methods have also been employed for urinary creatinine and urea. Parke, Davis & Company's solution of adrenalin chloride, 1 to 1000, has been used.

Experimental. In all, 128 injections have been given, 1 cc. being the usual dose. Fifty-five were subcutaneous injections, with blood sugar rises in every instance and with blood urea rises such as those given in table 1 in the 12 experiments in which these determinations were made.

One intramuscular injection resulted in typical blood sugar and urea rises, the time intervals being shorter than following subcutaneous administration. Sixty-nine injections were made through the abdominal wall with sugar rises in 49 instances, the blood urea also showing increases in the 12 rabbits in which the blood chemistry was followed for 6 or more hours. The rises were more rapid than with intramuscular or subcutaneous injection. Since 20 of the intra-abdominal injections resulted in no blood sugar or urea changes, the possibility that the adrenalin was being put into the lumen of the intestine instead of the peritoneal cavity at once

TABLE 1

Comparative blood analyses showing the similarity in blood chemistry after corpus luteum and subcutaneous and intraperitoneal adrenalin injections

TIME	CORPUS LUTEUM			ADRENALIN			ADRENALIN		
	Lactating doe 114 6/3/30			Normal doe 109 5/29/30			17 days pregnant doe 110 5/27/30		
	Sugar	N.P.N.	Urea	Sugar	N.P.N.	Urea	Sugar	N.P.N.	Urea
	mgm./ 100 cc.	*mgm./ 100 cc.*	*mgm./ 100 cc.*	*mgm./ 100 cc.*	*mgm./ 100 cc.*	*mgm./ 100 cc.*	*mgm./ 100 cc.*	*mgm./ 100 cc.*	*mgm./ 100 cc.*
Before injection	113	25.5	18.0	124	20.3	11.4	110	21.7	12.5
	Intraperitoneal injectionof 0.4 cc. corpus luteum			Subcutaneous injection of 1 cc. adrenalin			Intraperitoneal injection of 1 cc. adrenalin		
1 hour after	260			308			322		
2 hours after	324			370			313	23.0	14.4
3 hours after	263	25.5	18.8	425	21.5	13.4			
4 hours after				290	21.4	14.3	116	27.0	16.7
5 hours after	144	31.0	20.0	203	21.0	18.9			
6 hours after				156	25.0	19.3	93.0	29.0	19.5
7 hours after	131	35.8	28.0						
8 hours after				122	28.5	19.8	99.0	26.2	15.2
9 hours after	116	32.8	23.8						
10 hours after				111	22.3	16.6			
Maximum rises...	211	10.3	10.0	301	8.2	8.4	212	7.3	7.0

suggested itself. Under ether a small incision was made through the abdominal walls of six rabbits and the wounds temporarily closed with adhesive plaster. On the following day adrenalin was injected directly into the lumen of both small and large intestine and caused no blood sugar or urea changes. This is in agreement with the general consensus that adrenalin is not effective when given in the gastro-intestinal tract. In the same animals administration of adrenalin through the wound into the peritoneum gave the typical changes.

From the above experiments it was concluded that subcutaneous and

intraperitoneal injections of adrenalin always resulted in blood sugar and blood urea rises such as those given in table 1, the only difference between the two being a slower rise after subcutaneous administration, undoubtedly due to slower absorption. The occasional lack of response to intra-abdominal injections was attributed to accidental puncture of the intestine.

In an effort to determine whether the blood urea rises were part of a catabolic phenomenon due to adrenalin or were associated with retention by the kidneys, several lines of experimentation were followed.

If the blood urea rises were due to kidney retention, it would be expected that a decrease in urinary urea and volume of urine would accompany the reaction to adrenalin; whereas the catabolic production of urea without kidney damage would have the opposite effect.

It is not easy to trace increased excretion of urea. As has been found by injection of urea into the blood stream, the elimination is very slow, sometimes spread over days. Furthermore, the amount of urea normally present in the urine is so great as compared with that in the blood that changes in the output are difficult to follow. Also, the difficulties in collecting rabbits' urine, either in a metabolism cage or by catheter, render the calculation of urea by volume inaccurate. In order to overcome this last source of error creatinine determinations were run on each specimen as a gauge of the concentration of the urine, and the urinary urea is therefore reported in urea to creatinine ratios; i.e., U/C signifies milligrams urea divided by milligrams creatinine per 1 cc. urine.

In four cases of single subcutaneous injections of adrenalin into normal bucks whose blood sugars and ureas were found to give typical curves, catheter specimens were taken before and from 5 to 8 hours after the injections No change was observed either in the concentration of the urine or in the urea output. These experiments indicated that there was at least no immediate demonstrable effect upon the kidney. It could not be expected that a 7 to 12 mgm. rise in the blood urea would be traceable in the urine.

The effect of repeated injections of adrenalin upon blood and urine urea was next tried. Four such experiments were run on four healthy buck rabbits. Five one-cubic-centimeter injections of adrenalin were given subcutaneously during the course of one day. The blood sugars and ureas and the urinary creatinines and ureas (on catheter specimens) were followed at intervals throughout that day and the three days following. The blood sugars were high after the first injection and throughout that day, but had returned to normal the next day, where they remained during the rest of the experiment. The blood ureas rose to more than three times the normal values on the day following the injections. There was no decrease in the volume of urine or in the urea excretion as indicated by the U/C ratios. Inasmuch as the blood urea values had returned to nor-

mal on the morning of the fourth day, while the animals were still fasting, the rises in blood urea cannot be attributed to starvation. In two of these experiments there was a suggestion of increased urea excretion during the afternoon and evening of the day following the injections. In none of the animals was there any indication of retention by the kidneys as would be presumably shown by increased concentration of the urine (i.e., decreased volume) and decreased urea excretion.

Three normal doe rabbits, kept in metabolism cages, were given 1 cc. of adrenalin daily for 14 days and the twenty-four-hour urines collected. The volumes and concentrations of the urines were remarkably constant and there was no evidence of increased or decreased urea excretion based on the U/C ratios.

In order to see if it were possible to demonstrate any kidney damage by maintaining the blood urea at a high level through frequent adrenalin injections, a normal doe rabbit was given twenty-two 1 cc. doses of adrenalin over a period of 8 days and the blood sugars and ureas followed at intervals during this time. Before the first injection the urea level was at 12.8 mgm. per 100 cc. During the second and third day the values were between 25.0 and 37.2 mgm. per 100 cc. It was interesting to note that after the third day the blood ureas did not rise as much following injections as they did earlier, the values being between 15.0 and 27.5 mgm. per 100 cc. If the kidneys were being damaged a piling-up of urea would be expected. On the morning of the ninth day, the last injection having been given at 10:00 a.m. the day before, the blood urea was back to 11.5 mgm. per 100 cc. The animal was kept in a metabolism cage throughout the experiment and the twenty-four-hour urines were collected with all possible precautions. Although the blood ureas were consistently high, the twenty-four-hour volumes of urine varied within normal limits (100 to 200 cc.). Furthermore, both the values for the U/C ratios and those for the twenty-four-hour excretion of urea indicated a slightly increased urea output during the third, fourth and fifth days. It may therefore be concluded that the kidneys were not affected. The figures for the twenty-four-hour creatinines suggested the possibility of an increased excretion of creatinine, but from the limited data at hand no conclusion may be made.

In the case of single injections of adrenalin (table 1) it was noted that the maximum rise in urea came after the sugar rise had subsided. It was thought possible that the catabolism of proteins, as indicated by the urea rise, occurred only after the readily available sugar had been exhausted. Intravenous glucose was therefore given to a normal doe rabbit three hours after the subcutaneous injection of 2 cc. of adrenalin, when the blood sugars would normally start down. By the repeated administration of glucose the blood sugars were kept between 300 and 450 mgm. per 100 cc. for the next six hours, 15 grams of glucose being given in all. In spite of this the

blood ureas rose during this period from 12.9 to 25 mgm. per 100 cc. and had returned to normal by the next morning. A marked diuresis was caused by the glucose injections and the urine excreted gave a strongly positive test for sugar. At another time this same rabbit was given the same amount of glucose at the same intervals without being given adrenalin. There was no change in blood ureas. The blood sugars rose to 340 mgm. per 100 cc. an hour after the first injection of glucose, but the subsequent injections never raised the sugar level above 140 mgm. per 100 cc. There was no diuresis in this experiment. These results suggested that injected glucose is more readily utilized in the normal animal than in the animal that has received adrenalin. It was thought possible that the urea rise might be associated with some such phenomenon. Two more experiments were run, therefore, in which insulin and glucose were both given during the six hours following the injection of 1 cc. of adrenalin. By this means the blood sugars were kept between 70.0 and 150 mgm. per 100 cc. throughout the experiment. In both cases the effect upon the blood ureas was an exaggeration of the usual rise following 1 cc. of adrenalin (the rise being three times the normal values) as well as a prolongation of the high urea level, the normal not being reached until 48 hours after the adrenalin was administered. Insulin alone was given to a rabbit in an amount sufficient to cause the same degree of hypoglycemia as in these experiments and was found to have no effect upon the blood ureas. These results with insulin and glucose would seem to indicate that increased utilization of glucose, with a consequent depletion of the available sugar, exaggerates the effect of adrenalin upon protein catabolism. The results with repeated adrenalin injections, however, and also the results of glucose alone with a single adrenalin injection showed that the highest level of blood urea is often accompanied by very high blood sugar values. This observation suggests the possibility that adrenalin causes a decreased utilization of sugar in relation to the supply, and that the urea rise may be associated with some such phenomenon.

Discussion. After the completion of the investigations so far described, a reference was found to the work of A. Marie in 1922, in which it was reported that the administration of adrenalin to rabbits resulted in a rise in blood urea (2). This was confirmed by other French workers at that time and found to apply to other animals. It is rather surprising that neither the text-books nor the reports of investigations into the action of adrenalin which have since been published make any reference to this paper. These later workers have been emphasizing the effect of adrenalin upon carbohydrate metabolism, but any influence upon nitrogen exchange in the body following adrenalin might also be an important aspect of the whole picture.

In a later paper (3) Marie states that he has had no indication of kid-

ney damage in his animals that received large doses of adrenalin. He does not, however, report any urine studies. Sollmann, in his latest text-book of pharmacology (4), gives references to early investigators (Addis *et al.*, 1918) who reported an increased urea excretion in rabbits after hypodermic injection of epinephrine. Sollmann states, however, that further work is needed to make the conclusions convincing. The results of the present investigation have convinced the writers that the rise in blood urea is a result of some catabolic action upon protein metabolism and that there is no retention by the kidneys.

Cori and Cori, in their recent papers on the mechanism of epinephrine action, conclude that the increase in liver glycogen observed after epinephrine is a result of conversion of muscle glycogen into liver glycogen with lactic acid as an intermediary stage. In their first paper (5) they suggested another possibility: that there is a "conversion of protein into liver glycogen with a simultaneous oxidation of the glycogen disappearing in the muscles; i.e., a combination of two processes, one anabolic and the other catabolic." In later papers, however, this explanation is discarded, and no attempt is made to observe possible changes in nitrogen metabolism. From the results given in the present report it would appear that some catabolic action upon proteins is involved and must be considered in studying the mechanism of the action of adrenalin.

Cori and Cori believe that decreased utilization of blood sugar in relation to the supply plays an important rôle in adrenalin hyperglycemia (6), (7), (8). It is possible that the urea rise may be a result of decreased utilization of glucose. However, our finding that the urea rise after adrenalin is exaggerated by the administration of insulin, which supposedly aids glucose utilization, does not support this hypothesis. Cori and Cori (8) find that although insulin prevents the hyperglycemia which normally follows the injection of adrenalin, it has little or no effect upon the rise in blood lactic acid. From this one would conclude that insulin does not influence the glycogen breakdown in the muscles which is brought on by adrenalin, but only counteracts the adrenalin hyperglycemia. Considering our own experiments in the light of this work of the Coris, the possibility suggests itself that there is some protein breakdown associated with the glycogen breakdown in the muscles after adrenalin and that neither of these effects is antagonized by insulin.

It is conceivable that if muscular activity were to follow the adrenalin, the blood chemistry picture would not be the same as it is under experimental conditions. In any case, both protein and glycogen breakdown in the muscles as a result of adrenalin stimulation would be entirely in harmony with Cannon's conception of adrenalin as an emergency endocrine that assists in the preparation for muscular contraction.

The writers by no means feel prepared, on the basis of the investigations

here reported, to present any new theory of the mechanism of adrenalin action. The finding of a marked influence of adrenalin upon the nitrogen exchange in the body would seem, however, to present new possibilities both in the interpretation of past investigations and in the stimulation of further work.

SUMMARY OF RESULTS

Intramuscular, subcutaneous and intraperitoneal injections of 0.1 mgm. doses of adrenalin result in marked rises in blood sugar and later in the urea of the blood.

There is no retention by the kidneys, as shown by urine analyses during prolonged experiments in which repeated injections of adrenalin were given. There is, in fact, some indication of an increased excretion of urea. It is concluded, therefore, that the rise in blood urea is a result of some catabolic action of adrenalin upon protein metabolism.

The intravenous administration of glucose after the injection of adrenalin does not affect the rise in urea.

The administration of glucose and insulin after adrenalin exaggerates and prolongs the urea rise.

The writers wish to express their gratitude to Prof. Otto Folin for his constant interest and valuable advice.

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CHANGES IN SUGAR AND LACTIC ACID CONTENT OF BLOOD CAUSED BY BURNS

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Underhill, Carrington, Kapsinow and Pack (1923), Davidson (1925), Greenwald and Eliasberg (1926) and others have found that the sugar content of blood is increased after severe burns. Weiskotten (1919) emphasized the lesions found in the suprarenal glands following burns, and Olbrycht (1924), Greenwald and Eliasberg (1926), and others have since attributed the increase in blood sugar to an increased production or liberation of epinephrine. Riehl (1928) removed one of the suprarenals of guinea pigs or cats before burning, and the second gland from one to nine hours after burning. By extracting these extirpated glands with physiologic salt solution and using epinephrine in control experiments, the conclusion was reached that the amount of epinephrine produced by glands of the burned animals was usually unaltered, and that the histological changes observed cannot account for the poisoning observed after severe burning. Since Cori and associates (1929, 1930) are able to show that high blood sugar values produced by injection of epinephrine are accompanied by a rise in the lactic acid values it would seem that a study of the blood sugar and lactic acid in burned animals should aid in the solution of the problem in which the two contending views just outlined exist. Furthermore if the high blood sugar values are due to an increased activity of the suprarenal glands, a similar study of burned animals from which the glands were removed would appear significant.

EXPERIMENTAL. Rabbits of about two kilograms body weight and in good nutritional condition were selected as experimental animals. The hair was removed from the back and sides by closely clipping. Animals were burned without fast, and after assurance that they had had access to food and water for several hours immediately preceding the burning. The burns were made with water which had been brought to boiling temperature, and the flame extinguished just before immersion. After ether anesthetization the animals were partially suspended in the hot water for thirty seconds. Suspension was made in such a manner that a roughly eliptical area covering the back and sides was immersed. The area of the

burned surface was estimated by calculating the area of the elipse. The percent of total body surface burned was estimated by use of the Meek (1879) formula for rabbit surface excluding the ears. The standing position was regained in from fifteen to thirty minutes after immersion. Blood samples were taken from the heart. The initial values were obtained

TABLE 1

Blood sugar and lactic acid (mgm. per 100 cc.)

0.5 HOUR		1.0 HOUR		1.5 HOURS		4.0 HOURS		24.0 HOURS	
Sugar	Lactic acid	Sugar	Lactic acid	Sugar	Lactic acid	Sugar	Lactic acid	Sugar	Lactic acid
192.3	24.6	307.7	19.9	256.4	24.2	327.9	12.9	555.6	23.8
246.9	24.8	224.7	19.0	188.7	21.9	289.9	11.9	186.9	13.4
162.6	14.1	224.7	26.8	259.5	19.0	217.0	16.7	224.7	14.7
277.8	19.3	229.2	22.0	181.2	23.2	384.6	16.7	190.3	9.2
		277.8	20.7	437.3	25.7	317.0	21.9	246.9	7.3
		263.3	21.3			307.0	19.3		
		250.0	15.9						
Average values									
219.9	20.7	253.9	20.8	264.6	22.4	306.2	16.6	282.9	13.7
Average initial values of the same animals represented above									
114.1	15.6	116.5	16.6	122.6	18.5	115.6	16.4	119.0	17.6

TABLE 2

Suprarenalectomy

INITIAL		1 HOUR		2 HOURS		3 HOURS	
Sugar	Lactic acid	Sugar	Lactic acid	Sugar	Lactic acid	Sugar	Lactic acid
115.0	21.3	270.3	25.8				
105.2	16.4	166.7	17.9	241.0	18.6	289.9	17.3
101.5	12.9	151.5	12.7	162.6	13.8	200.0	13.1
99.5	13.3	163.9	12.9	165.3	15.1	154.9	15.9
101.5	17.8	277.4	18.2	257.1	18.3		
Averages							
104.5	16.3	205.9	17.5	206.5	16.1	214.7	15.4

from blood taken before anesthetization. Folin's micro-method (1928) was used to determine the sugar, and the lactic acid determinations were made by the Friedemann and Kendall (1929) modification of the Friedemann, Cotonio and Shaffer method. Since the animals were not able to endure the combined strain of the burn and repeated heart puncture at the short

intervals desired it was found necessary to omit collections at some periods and thus stagger the time of collections. The results are given in table 1. Because of this necessary staggering the sugar and lactic acid values in the same horizontal lines do not represent successive values for the same animals and are therefore not comparable to each other. The sugar and lactic acid values for a given time are however the results of analysis of the same sample. A total of seventeen animals is represented in the data shown in table 1. The average area burned was 21.0 per cent, the maximum area was 27.8 per cent and the minimum 16.8 per cent.

Similar data were collected from a second group of rabbits from which the suprarenals were removed by bilateral suprarenalectomy. Removal of the glands and burning were carried out during the same anesthetization. In this experiment the blood collections were made at the end of the first, second and third hours after burning. Initial values were obtained from blood collected before anesthetization. Post-mortem examinations were made for suprarenal tissue, and none was found in the five animals included in this report. The results are given in table 2.

Discussion. Burned rabbits are very lethargic. They sit huddled and without movement for hours, and offer little or no resistance during blood collections. It will be seen by examination of table 1 that both the blood sugar and lactic acid values have increased. When the initial averages of lactic acid content of the same animals from which subsequent collections were made, are considered as average initial concentrations, the average lactic acid values for the five periods, in per cent of initial values are, 132.1, 125.3, 121.1, 101.0 and 77.8 in the order of time of collection. Thus the lactic acid content appears to rise quickly and rather slowly decreases, until at the end of the fourth hour it has returned to the initial. At the end of twenty-four hours the lactic acid is approximately but three-fourths of the initial value. These values are of the same order as those found by Cori and Buchwald (1930) when continuous intravenous injections of 0.00005 to 0.0001 mgm. epinephrine per kilogram body weight per minute were made, i.e., the minimum rate of infusion which is capable of influencing the carbohydrate metabolism. The same authors report evidence of a rapid destruction of epinephrine, thus both their lactic acid and sugar values fall rapidly during the first hour after stopping the injection and have returned to the initial values at the end of the second hour. In as much as the values given in table 1 show a return to the initial at the end of the fourth hour, and at the end of twenty-four hours are below the initial, it would seem that the period of increased activity of the suprarenals is limited to a short period immediately following the burning, or the rate of conversion of the acid to glycogen is accelerated. The latter possibility seems very unlikely because of the low blood pressures found after burns. A similar treatment of the average blood sugar values of table 1 shows that the in-

creases are to 189.3, 217.9, 215.7, 264.9 and 237.7 per cent of the average initial values for the same animals. These are of the order of continuous injection of 0.0001 to 0.0005 mgm. epinephrine per kilogram body weight per minute, as reported by Cori, Cori and Buchwald. This appears to be greater than can be accounted for by the epinephrine output as indicated by the lactic acid values. Furthermore the maximum average blood sugar content shown is not reached until three and one-half hours after the maximum lactic acid and in case of survival remains high for twenty-four hours when the lactic acid has returned to a value below the initial. This continued rise might be considered as due to accumulation of sugar resulting from a decreased rate of oxidation when epinephrine is administered continuously, as shown to occur by Colwell and Bright (1930), were it not that their data indicate that the oxidative power is partially recovered within one hour after the injections are stopped, and is nearly restored in four hours. Furthermore Cori and Cori (1929) have shown that in single large subcutaneous injections of epinephrine the maximum lactic acid content is reached in one hour but the maximum sugar values do not appear until the second hour and decrease during the third and fourth hours as the lactic acid content decreases. Here the sugar values continue to rise three hours after the lactic acid values begin to fall, and continue high after twenty-four hours when the lactic acid content is below the initial value. That a retarded blood circulation rate does not account for the high sugar content would seem to follow from the evidence that the removal of the lactic acid proceeds at a nearly normal rate.

The data shown in table 2, collected from the blood of burned animals previously suprarenalectomized likewise show sugar values which appear too high to be accounted for on the basis of the lactic acid intermediate. The sugar values expressed in percent initial are nearly as great as those in table 1. Lactic acid values however do not show the increase seen in the first collections from the animals that were permitted to retain the glands. It is suggested that the sluggish circulation may be a factor in retaining the initial acid values for the three hour period.

SUMMARY

Studies have been made of the concentration of sugar and lactic acid in the blood of rabbits, burned with the suprarenal glands intact, and after the removal of the glands. The concentrations of both sugar and lactic acid were found to be increased. The increases of lactic acid were small and do not appear to account for the high sugar values. Twenty-four hours after burning, animals with the intact glands were found to have maintained the increased blood sugar while the lactic acid concentrations were reduced below the initial values. The studies indicate that increased activity of the suprarenals do not account for the increased blood sugar through lactic acid as an intermediate, or by a decreased rate of oxidation of the sugar.

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THE INNERVATION OF THE RENAL PARENCHYMA

A Study to Demonstrate Nerve Endings in Renal Epithelium

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There has been little reference in the literature to the study of the influence of the innervation on the function of the kidney. It has always been assumed that the only influence of the nervous system is through the circulation. This explanation would be satisfactory if no other nerve fibers were present than those of the blood vessels.

If however definite anatomical proof of a nerve supply to the secreting tubules should be demonstrated it would be reasonable to assume that it should exist for some function. Furthermore there are disturbances of renal function which are difficult to account for on a basis of a purely vascular disturbance.

It is a well known fact, first demonstrated by Pappenheim (1), that together with the renal arteries autonomic nerve fibers enter the kidney. He was able to follow them as far as to the arteries of a caliber of 0.03 mm. Koelliker (2) and Retzius (3) could follow them to the finest capillaries. They found the nerve fibers forming plexuses innervating the vascular musculature. These plexuses end on the glomeruli. Disse (4) was the first to demonstrate nerve fibers in kidneys of mice which reached the tubules and ended on them. He was able to show with the Golgi-method two to three fibers accompanying a tubule. From these nerve fibers branches came off at right angles, crossing the tubules and connecting the longitudinal fibers, thus forming plexuses. These plexuses ended near the glomerulus. The longitudinal fibers eventually branched off into very fine radicles, which ended with small nodules, some of the endings reaching the capsule of the glomerulus. He could however not decide whether the nerve fibers ended in the epithelium of the tubules or on the membrana propria.

A more detailed description was given by Smirnow (5). He also found nerve fibers entering together with the arteries and from them branches reached the tubules forming plexuses around them. The branches from these plexuses ended partially on the membrana propria, and partially entered the epithelium, ending between the epithelial cells. The methods employed by Smirnow were Golgi's method and Ehrlich's intravital meth-

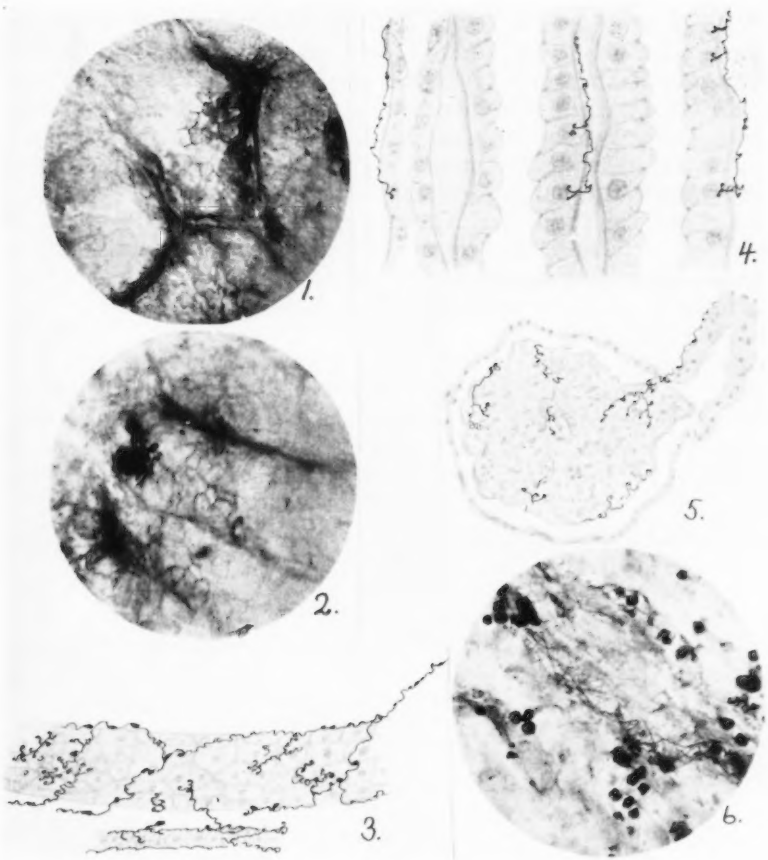

Fig. 1. Microphotograph. Human kidney. Methylene blue. Nerve fibres around tubules.

Fig. 2. Microphotograph. Human kidney. Methylene blue. Nerve fibres around tubules.

Fig. 3. Drawing of a section through a human kidney. Modified Bielschowsky. Piece of a tubule with a capillary. Nerve endings are seen on the membrana propria.

Fig. 4. Drawing of a section through human kidney. Modified Bielschowsky. Nerve fibres between the tubules with nerve endings in the epithelium.

Fig. 5. Drawing of a section through a human kidney. Modified Bielschowsky stain. A glomerulus with nerve endings.

Fig. 6. Microphotograph of a section through a dog's kidney. Intravital methylene blue stain. Photograph of a nerve plexus aroung a tubule.

ylene blue method. The objects studied were kidneys of embryos and adults of many kinds of mammals. His observations on human kidneys however were few and incomplete. In spite of these anatomical findings no step forward has been made in the physiological function of these nerve fibers.

It seemed necessary therefore to us not only to repeat and corroborate these observations on nerve fibers in animals, but also to study their distribution and endings in the human kidney. We have studied sections of kidneys from rats, guinea pigs, cats, dogs, and man.

Technique. The methods for staining nerve fibers are unfortunately very unreliable, especially in organs like the kidney which are rich in connective tissue. Experimenting with a great number of staining methods we found two methods satisfactory; the intravital methylene blue method (is the better) and Bielschowsky's silver method as practiced in the McGill Pathological Institute (6). The intravital method was used in several ways. In smaller animals a canula was introduced into the jugular vein and a one-eighth of one per cent methylene blue solution in normal saline was allowed to run in slowly while one of the femoral arteries was opened allowing the circulation to be completely washed out with the methylene blue solution. In larger animals and human kidneys, removed by operation, the methylene blue solution was directly injected into the renal artery and the whole vascular system of the kidney washed out with it.

Small pieces of the organ, about 3 mm. in thickness, were then removed and placed into a flat dish with glass wool which was previously moistened with one quarter of one per cent methylene blue solution in normal saline and the pieces were kept in an incubator at a temperature of 37°C. for two hours, air having access, which is essential for staining. The stain was then fixed in a 7 per cent ammonium molybdate solution for 8 to 10 hours and the pieces then hardened for one hour in 10 per cent formalin. It is impossible to harden the tissue longer since the stain is easily affected. The pieces were then cut on the freezing microtone.

In human kidneys stained by the modified Bielschowsky (6) method it is essential that the material should be fresh and only kidneys have been employed which were removed within six hours after death.

Findings. The nerve fibers innervating the tubules seem all to originate from the nerve fibers accompaning the arteries to these tubules. In other words, tubular nerve endings and arterial nerve endings are derived from the same nerve trunk. They are all non-medullated fibers and are found in the cortical as well as in the medullary substance of the kidney. These fibers form plexuses on the membrana propria, from these plexuses fine varicose branches come off forming nerve endings. Some of these endings are on the membrana propria itself, while other branches enter the tubules to end between the epithelial cells.

The types of endings which we were able to see were threefold, nodular,

brush-like, and arborized. These can be studied in the accompanying plates. These nerve fibers, plexuses, and nerve endings were found on a considerable number of tubules in many of the sections. Since however in a great number of tubules nerve fibers could not be demonstrated, there arises the question, whether the innervation of the tubules is a uniform and general one or whether only some of the tubules are innervated. Although we can not answer that question definitely we feel inclined to believe that the absence of nerve fibers in a great number of the tubules is due rather to deficiencies in the methods than anything else. We believe that further perfection of the methods may yield a greater percentage of positive findings than is at present available.

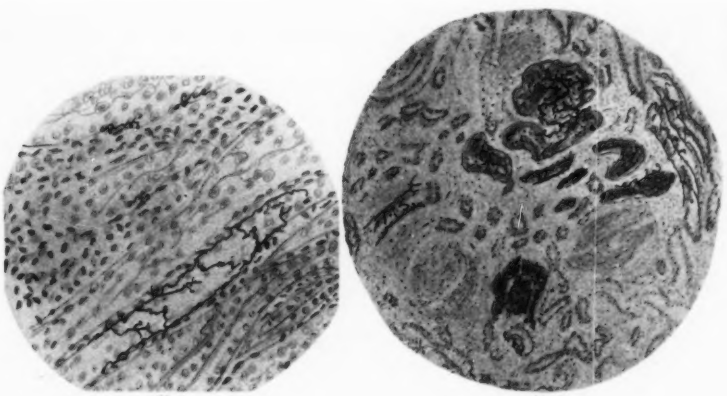

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Fig. 7. Retouched photograph. Human kidney. Modified Bielschowsky. Nerve fibres around tubules.

Fig. 8. Retouched photograph. Human kidney. Methylene blue. Nerve fibres around tubules.

In regard to the nerve fibers of the blood vessels nothing new is to be added. The fibers form dense plexuses in the adventitial and medial layers of the arteries and veins; nerve endings were also seen. These fibers could be followed to the capillaries, ending in the glomeruli. The fibrous capsule of the kidney shows a rich nerve supply. The nerve fibers run together with the blood and lymph vessels. They are medullated as well as non-medullated. Occasionally fibers are seen to branch off into the fibrous tissue.

The description of nerve fibers, plexuses, and nerve endings as given above holds true for the mammalian kidney in general.

From our findings we feel we are justified in arriving at the following

conclusion, namely, that an extensive non-medullated innervation of the parenchyma of the kidney is present in addition to the purely vascular nerve supply already described.

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THE TEMPORARY CONTROL OF POST-OPERATIVE TETANY IN THYRO-PARATHYROIDECTOMIZED DOGS BY THE ADMINISTRATION OF THYROID HORMONE[1]

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The possibility of a functional relationship existing between the thyroid and parathyroid glands has frequently been indicated from reports of definite changes provoked in either of these glands by a disturbance in the functional activity of the other. These reported changes consist in hypertrophy of the external parathyroids after removal of the thyroid and internal parathyroid, hypertrophy of the parathyroids after thyroidectomy, atrophic changes in the thyroid following transplantation of the parathyroids in rats, and structural changes in the parathyroid glands in clinical cases of hyperthyroidism. In addition to these observations, numerous references to clinical material, beginning with Kocher's practice, indicate that apparently beneficial effects have been obtained in cases of post-operative tetany by the administration of large doses of thyroid substance. Individual references to the above-mentioned articles are not cited inasmuch as this literature has been recently reviewed by McCullough (1928) in his publication of the thyroparathyroid relationship in a large number of cases of diseases of the thyroid gland.

If the thyroid hormone, to a demonstrable degree, controls the metabolic processes regulated by the parathyroid glands, experimentally-induced hyperthyroidism should influence the symptom complex of post-operative parathyroid tetany, as well as the characteristic changes in the calcium and the acid soluble phosphorus of the blood, following extirpation of the parathyroid glands. With the view of obtaining some evidence on these points, the following experiments were performed.

METHOD AND RESULTS. Dogs weighing 10 to 12 kilos were thyro-parathyroidectomized according to the usual technique. Two series of experiments were performed:

1. The preliminary series, in which only 5 dogs were used. These animals received no thyroid substance previous to the extirpation of the

[1] This work has in part been conducted under a grant from the Douglas Smith Foundation for Medical Research at the University of Chicago.

[2] Preliminary report, This Journal, 1928, lxxxv, 387.

TABLE 1

Data showing the effect of experimental hyperthyroidism on the serum calcium and acid soluble phosphorus of the blood in dogs before and after thyro-parathyroidectomy

DOG	PRE-OPERATIVE					POST-OPERATIVE						THYROID FEEDING		DEATH OCCURRED		REMARKS
	Normal		After thyroid feeding			Thyroid feeding continues			Thyroid feeding discontinues			Pre-operative days (Amounts)	Post-operative days (Amounts)	After discontinuing thyroid	After operation	
	Ca	P	Ca	P	Days	Ca	P	Days	Ca	P	Days					
														days	*days*	
1	None	None	None	None	None	7.0	5.2	8	8.5	6.3	5	0	21 (10 gm.)	19	40	Died in profound depression. No symptoms during hyperthyroidism
						9.1	7.4	15	6.5	6.5	12					
						8.1	10.7	21								
2	11.3	3.8	11.0	5.0	4	9.4	5.2	3	5.8	5.3	5	5 (10 gm.)	11 (10 gm.)	9	21	Depression
						7.9	7.3	11	5.4	5.5	12					
3	None	None	None	None	None	None	None	None	None	None	None	14 (5 gm.)	6 (5–10 gm.)	0	6	Tetany
4	None	None	None	None	None	5.4	10.0	19				14 (5 gm.)	21 (10 gm.)	4	25	Death from snuffles. No tetany. No depression
5	None	None	None	None	None	5.6	5.8	19				14 (5 gm.)	21 (10 gm.)	2	23	Slight twitching beginning 2 days after operation and continuing till death
6	None	None	None	None	None	10.2	6.0	20	8.5	6.5	4	14 (5–10 gm.)	21 (10 gm.)	13	34	No symptoms until 4 days after discontinuing thyroid. Then depression and tetany
									5.4	7.0	7					
									5.4	6.3	13					
7	None	None	None	None	None	7.0	7.5	20	6.2	7.0	4	14 (5 gm.)	21 (5 gm.)	540	562	Severe tetany 3 days after thyroid was discontinued; then lasted about 15 days
									5.8	7.1	7					
									5.5	5.7	13					
									6.1	8.4	35					
									8.5	7.5	48					
									7.3	7.6	56					
									7.2	7.3	61					
									6.0	7.2	5 mos.					
									5.5	7.0	9 mos.					

8	11.6	3.2	11.3	4.9	8	6.0	8.3	18				7 (10 gm.)	21 (10 gm.)	2	23	Slight tremor. No convulsions. Pneumonia
9	11.1	4.1	11.0	9.4	7							7 (10 gm.)	11 (10 gm.)	0	11	Snuffles
10	10.6	5.0	10.8	6.1	19	7.0	9.3	2				14 (5 gm.)	2 (5 gm.)	0	2	Tetany
11	10.8	3.7	10.6	3.8	19	8.0	6.5	7				14 (10 gm.)	14 (10 gm.)	0	14	Tetany and pneumonia
12	10.6	3.0	10.6	3.5	19	9.1 7.4	5 8.5	7 28	6.6	10	4	14 (10 gm.)	28 (10 gm.)	4	32	Tetany
13	10.9	3.6	11.6	6.3	13							13 (2½ mgm.)	2 (2½ mgm.)	0	2	Violent tetany
14	10.4	3.2	no	3.9	13							13 (2½ mgm.)	2 (4 mgm.)	0	2	Violent tetany
15	10.2	3.0	10.4	3.4	11							10 (2½ mgm.)	2 (4 mgm.)	0	2	Death due to violent tetany
16	11.1	3.7	11.4	7.1	8	7.5	8.7	4				10 (3 mgm.)	15 (6–8 mgm.)	0	15	Tetany
17	10.6	4.1	10.6	6.1	6	8.8	7.8	4	6.3	8.0	4	10 (6 mgm.)	28 (6–8 mgm.)	4	31	Entirely free from symptoms until thyroid was discontinued
18	10.2	3.8	10.6	4.1	9	8.0 7.3 7.3 6.6	5.5 8.0 8.2 7.8	6 14 21 28	5.2 5.09 5.5 5.1	7.5 5.7 6.0 5.8	5 13 mos. 15 mos. 17 mos.	10 (2½–4 mgm.)	28 (4–8 mgm.)	630	658	Slight tetany and depression for 5 days after discontinuing thyroid
19	10.5	3.7				7.6	5.3	8				7 (10 mgm.)	11 (5–10 mgm.)	0	11	Tetany
20	10.4	5.1	10.2	5.5	4							4 (5 gm.)	2 (10 gm.)	0	2	
21						5.6	5.8	23				14 (5 gm.)	21 (5–10 gm.)	1	23	Trembly and slightly spastic
22	9.0	5.0										4	3 (10 gm.)	0	3	Tetany
23	10.6	5.0	10.8	6.1	19	7.0	9.3	2				14 (50 gm.)	2	0	2	
24	9.6	3.2	9.8	5.4	7	5.0	7.8	7				7 (6 mgm.)	7	0	7	Tetany 2 days following operation

TABLE 1—*Concluded*

DOG	PRE-OPERATIVE					POST-OPERATIVE						THYROID FEEDING		DEATH OCCURRED		REMARKS
	Normal		After thyroid feeding			Thyroid feeding continues			Thyroid feeding discontinues			Pre-operative days (Amounts)	Post-operative days (Amounts)	After discontinuing thyroid	After operation	
	Ca	P	Ca	P	Days	Ca	P	Days	Ca	P	Days			*days*	*days*	
25	10.5	3.7	10.7	4.6	9.0	5.4	7.2	6				9 (10 mgm.)	6	0	6	Tetany 4 days after operation
26	12.3	3.8				No analysis	8.6	7.0				13 (6-9 mgm.)	8	0	8	Tetany
27	10.6	3.9	11.1	4.1	9.0	9.5	8.3	5	5.4	5.0	9	8 (6 mgm.)	21 (6 mgm.)	9	32	Tetany
28	10.9	3.9	11	6.5	9.0	7.0	8.9	5	6.9 10.7	8.0 5.0	14 73					Accessory Parathyroid
						8.4	7.4	11	10.0	3.6	26.1					
29	10.4	3.4	10.3	4.2								7 (6 mgm.)	6	0	6	Tetany 2 days following operation
30	10.5	3.5	10.4	4.7	8							8 (6 mgm.)	6	0	6	Tetany 2 days following operation
31	10.3	4.3	10.2	4.6	5							5 (6 mgm.)	3	0	3	Tetany

thyro-parathyroid glands. Following the operation, each dog received 5 to 10 grams of desiccated thyroids[3] daily. Four of these animals died as in uncontrolled tetany and are not included in table 1. The remaining is dog 1, of table 1.

2 In the seond series, 30 dogs were used (table 1, nos. 2 to 31 inclusive). Each of these received either desiccated thyroids per os, or thyroxin (Harrington and Barger, 1927) intravenously, for a period of time varying from 5 to 14 days preceding the operation (period of pre-operative preparation). This pre-operative thyroid medication was instituted to make sure that experimental hyperthyroidism (Kunde, 1927) had been induced before the onset of the usual post-operative parathyroid deficiency. The post-operative time is subdivided into two periods: *1.* The period of post-operative hyperthyroidism, during which an attempt was made to control the tetany by continuing the induced hyperthyroidism. This period varied from 2 to 28 days and terminated either by death of the animal or by discontinuing the thyroid substance. *2.* The period of uncontrolled tetany. During this time the ingestion of thyroid substance was discontinued and no attempts made to control the parathyroid deficiency (excepting instances in the tetanoid condition of dogs 7 and 18). This period varied from 0 to 630 days.

Beginning with the period of pre-operative preparation and continuing through the post-operative hyperthyroid period, the daily dietary of each dog contained at least 200 grams hashed, raw, lean meat; 250 cc. of milk and bread and water *ad libitum*. This dietary was also offered them during the period of uncontrolled tetany but was often refused. With the onset of hyperthyroidism, and continuing until the discontinuance of the thyroid medication, each dog received at least 500 cc. water by stomach tube in addition to the amount taken *ad libitum*.

Six control dogs, receiving the same dietary as the experimental animals, were carried along, at various times, through the surgical procedure, but receiving no thyroid substance at any time. Twenty to 48 hours after thyro-parathyroidectomy these dogs all showed the usual signs of acute loss of parathyroid function, fatal parathyroid tetany intervening 1 to 4 days later. Data from these animals are not tabulated.

Chemical analyses of the serum calcium (Tweedy modification of Kramer and Tisdall method, 1929) and the acid soluble phosphorus of the blood (Fisk and Subbarow, 1925) were made at different periods as indicated in table 1.

Discussion. The influence of the experimental hyperthyroidism on the post-operative tetany of these dogs varied widely. Despite this, analysis of the data seems to indicate that the results lend themselves to

[3] Armour's U. S. P.

classification into three distinct groups: Group 1 includes animals 3, 10, 13, 14, 15, 20, 22, 23, 24, 25, 29, 30 and 31. In all dogs of this group, fatal post-operative tetany occurred 2 to 7 days after extirpation of the parathyroid glands (see table 1). It is evident from this that in animals of this group, the hyperthyroidism had no demonstrable effect on the course of the parathyroid tetany. Group 2 includes dogs 1, 2, 6, 7, 9, 11, 12, 16, 17, 18 and 27. These animals lived 11 to 658 days after removal of the parathyroid glands. The post-operative period of hyperthyroidism varied from 11 to 28 days. During this time there was very little or no evidence of parathyroid deficiency. There is an increased greediness for food. Thirst is pronounced and the elimination of large quantities of urine and soft stools occurs daily. The serum calcium, in most instances, decreased less than 3 mgm. during the first post-operative week, but in the dogs with post-operative hyperthyroidism continuing into the third and fourth weeks there is farther lowering of the calcium as the period progresses. The acid soluble phosphorus is markedly elevated throughout the period, showing a general tendency to increase steadily as the time advances.

After discontinuing the ingestion of thyroid substance, some of these dogs appeared entirely normal for as long as 6 days. Then, without exception, there occurs either severe convulsions terminating fatally 1 to 3 days later, or apathy resulting in pronounced depression and a more chronic course of the tetany. Without exception the serum calcium decreases still farther, and in most instances there is a lowering of the acid soluble phosphorus. The salient points from the protocols of dogs of this group are found in the data of table 1, excepting dogs 1, 7, and 18. There unusual findings seem to warrant a more detailed description. Dog 1 belongs to the preliminary group and received no pre-operative thyroid. It is the only animal showing a post-operative drop in the serum calcium followed by a subsequent rise. This may be explained by the fact that on the 8th post-operative day the most favorable hyperthyroid influence was not operating because of the time element involved (Kunde, 1927) but was in effect on the 15th day. During the entire three weeks of post-operative hyperthyroidism this dog was entirely free from the signs of post-operative tetany, notwithstanding the lowered blood calcium. The first appearance of parathyroid insufficiency occurred five days after discontinuing the thyroid and 26 days after thyro-parathyroidectomy. This manifested itself in apathy which progressed until the 40th post-operative day, when the dog died in profound depression. Dogs 7 and 18 survived for a much longer period (approximately 18 and 21 months respectively). These dogs were in moderate tetany for about 2 weeks after discontinuing the thyroid treatment. No treatment of any kind directed towards the control of the tetany was given them during these 2 weeks, but they

improved markedly so that they appeared quite normal for many weeks thereafter. Then with an irregular periodicity the latent tetany would increase in severity so that each dog received control treatment symptomatically (usually for 2 to 3 days) after which the treatment was discontinued and they would again appear quite normal under ordinary laboratory routine for several weeks. Numerous analyses of the calcium and phosphorus during these intervals of no treatment indicate that the calcium never spontaneously increased above approximately 6 mgm. per 100 cc. blood, and the acid soluble phosphorus constantly remained elevated 60 to 100 per cent or more above the normal levels. During the intermittent tetany control days, dog 18 was given parathormone symptomatically (usually only 3 or 4 successive days) and died of parathormone overdosage 21 months after discontinuing the thyroid treatment. Dog 7 received calcium lactate (Luckhardt, et al., 1921–22) symptomatically and died in tetany, precipitated by discontinuing the calcium lactate, which at that time had been administered daily over a period of several weeks. This was 18 months after discontinuing the thyroid therapy. There is no evidence that these dogs (both were males) became more completely adjusted to the latent tetany as time progressed. On the contrary, the chronic tetany seemed more and more difficult to control. The periodic recurrence of tetany of increasing severity in male dogs under approximately the same dietary regime is also of interest.

The third group, dogs 4, 5, 8, and 20, is demarcated by the fact that during the period of post-operative hyperthyroidism, the serum calcium is well below the tetany level, and the acid soluble phosphorus higher than the calcium. Twitching and spasticity are constantly in evidence after the second or third post-operative day, but no severe convulsions. The hyperthyroid period terminated on the 21st day after operation and death followed 2 to 4 days later.

The findings in these three groups clearly indicate that the beneficial effects of hyperthyroidism, on the course of tetany in some dogs, cannot be due to the hyperthyroidism *per se* since this was apparent at operation in all dogs tabulated (excepting dog 1 of the preliminary group), and yet 45 per cent of them died as in uncontrolled tetany. The degree of hyperthyroidism present may have an important bearing on the results. Our data indicate that very severe hyperthyroidism is less effective than a more moderate degree of the same condition. For example, in the chemical analysis of the serum calcium made of dogs 1, 2, 12, 17, and 18 during the first, second, third and fourth weeks of the control period (28 cannot be included here because evidence of functioning accessories is apparent) without exception the calcium decreases whereas the severity of the hyperthyroidism increases as the period progresses. Moreover, in the second group, where twitching and spasticity were in evidence on the second and third post-operative day, in some instances the daily amount of

ingested thyroid substance was then doubled but with no evidence of a more ameliorating effect on the tetany than the moderate amounts. It is possible that a certain degree of hyperthyroidism effectively stimulates a mechanism capable of compensating for a time, at least, for the loss of the parathyroids. But as the toxicity of the hyperthyroidism increases, this mechanism may become overwhelmed and incapable of physiological response. Or perhaps in some dogs the mechanism is refractory to all degrees of hyperthyroidism. The possibility of a time element involved in breaking down a mechanism capable of response for a short while only, must also be considered. The relative importance of a time element and increased severity of hyperthyroidism cannot be established from the data at hand.

When correlated with previous investigation (Falta, 1916) our results suggest that the thyroid mechanism is closely associated with the mobilization and excretion of the calcium and acid soluble phosphorus of the blood, and that in some respects, experimental hyperthyroidism causes changes in these substances similar to the effects of subcutaneous administration of parathormone. In other respects, opposite results are in evidence, viz.: In the normal dog with the thyro-parathyroid mechanism intact, the production of moderately severe hyperthyroidism has no effect on the serum calcium (see table 1, pre-operative data), whereas parathormone causes a pronounced elevation in the level of the serum calcium (Collip, 1925). The concentration of the acid soluble phosphorus of the blood on the other hand may be increased by 100 per cent or more in moderately severe hyperthyroidism, whereas in hypercalcemia it remains at the normal level until fatal symptoms are in evidence. Parathormone causes a marked increase in the elimination of calcium in both feces and urine (Greenwald, 1925). Falta states that thyroid gland secretion markedly increases the elimination of phosphorus and of calcium through the intestine. After parathormone the urinary excretion of phosphorus may increase over 100 per cent, the excretion in the feces being less marked (Greenwald, 1925). From this it is evident that relative to the calcium, parathormone causes a disturbance both in the mobilization and in the mechanism which maintains it at a constant level in the blood serum, whereas with the phosphorus the disturbance is apparent only in the mobilization, the concentration in the blood remaining normal. This is due, according to Greenwald (1925), to effective elimination by the kidney. In hyperthyroidism experimentally induced there is a disturbance in the mobilization of the calcium indicated by increased elimination from the intestine (Falta, 1916), but the mechanism for maintaining the normal level in the blood serum is unimpaired (table 1, pre-operative data).[4]

[4] Data not included in this publication indicate that in unoperated dogs very severe hyperthyroidism of several weeks' duration may cause an increase in the blood serum calcium.

The acid soluble phosphorus in this condition shows disturbance in both the mobilization (increased elimination from the intestine) and the mechanism for maintaining a constant level in the blood serum. Why the kidney should effectively eliminate the phosphorus in hypercalcemia and fail to do so in hyperthyroidism is an extremely interesting fact which needs further investigation,

After parathyroidectomy in approximately 55 per cent of the dogs in our series, it is possible that the increased mobilization of calcium caused by the induced hyperthyroidism is capable of partially compensating for the decrease in calcium mobilization following sudden loss of the parathyroids (Greenwald, 1925). Failure to do so in the remaining animals indicates to us that important mechanisms other than the mobilization of calcium are involved. These have been duly discussed by Dragstedt (1927).

SUMMARY

1. In approximately 55 per cent of a series of 31 dogs, tetany following thyro-parathyroidectomy was temporarily controlled by experimental hyperthyroidism. The remaining 45 per cent died as in uncontrolled tetany.

2. Experimental hyperthyroidism maintained the serum calcium above the tetany level for 1 to 3 weeks in 55 per cent of a series of 31 thyro-parathyroidecomized dogs. In all thyro-parathyroidectomized dogs of this series, a farther decrease in the serum calcium followed the discontinuance of thyroid ingestion.

3. In the unoperated animals, moderately severe hyperthyroidism, experimentally induced, causes an elevation in the level of the acid soluble phosphorus of the blood serum which may reach 100 per cent above normal, with no change in the serum calcium. After thyro-parathyroidectomy, the increased concentration of the blood phosphorus persists, or shows a tendency to a still higher elevation with increased severity of the hyperthyroidism, regardless of the decrease in the serum calcium.

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THE ACTION OF ATROPINE AND ADRENALINE ON GASTRIC TONUS AND HYPERMOTILITY INDUCED BY INSULIN HYPOGLYCEMIA

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Bulatao and Carlson (1) found that dogs in a state of hypoglycemia induced by insulin exhibited increased gastric motility. From 10 to 60 minutes after the injection of 20 to 40 u. insulin subcutaneously, their animals developed increased hunger contractions, a rise in tonus of 1 to 4 cm. in water maonometer controls; and finally gastric tetany. The intravenous administration of glucose during this hyperactive period, if given in the presence of insulin, was followed by decreased motility, inhibition of tetany, and fall in gastric tonus. The administration of glucose in depancreatized animals did not inhibit the hyperactive stomach nor alter its tonus. When insulin was given with the glucose, inhibition of gastric contractions and lowering of tonus followed. The explanation of these findings was based on an hypothesis of "change in the energy metabolism of the gastric motor tissues when the available glycogen is reduced or sugar cannot be oxidized." It is suggested by the authors, however, that the gastric motor nerves may be concerned with the hyperactivity of hypoglycemia.

Greisheimer and others (2) have shown that the irritability of reflex arcs in decerebrate dogs increases as the blood sugar is lowered following insulin. The question, therefore, arises whether the increased motility reported by Carlson is due to an effect directly on the gastric muscle cells or to hyperactivity of the motor nerve (vagus) to the stomach.

McCrea, McSwiney, and Stopford (3), working with cats and dogs, have shown that stimulation of the peripheral end results in initiation of contractions and rise in tonus if the stomach is quiescent. The active stomach responds to vagus stimulation by inhibition for a short period followed by augmentation of movements previously present. They have shown (4) that stimulation of the peripheral end of the splanchnic nerves to the active fasting stomach or to a stomach actively contracting as the result of vagus stimulation produced a lowering of tonus and complete inhibition of movement.

It is known that atropine inhibits the vagus nerve, while adrenaline

acts as though the sympathetic nerves (in the case of the stomach, the splanchnics) are stimulated.

Correlating these known facts, the present problem has been to test the action of atropine and adrenaline on the nerves to a stomach brought into extreme hypermotility by hypoglycemia due to insulin injection.

It was thought that atropine by inhibition of the vagus would cut off motor impulses to the stomach, and therefore, if the hypermotility and increased tonus present in the state of hypoglycemia were due to increased vagus irritability, one would expect the hypermotility to be decreased and the tonus to be lowered by the giving of atropine. The splanchnics are inhibitory in the case of the active stomach and, therefore, their stimulation by adrenalin should produce inhibition exhibited by a decreased hypermotility and lowered tonus.

EXPERIMENTAL. A normal dog was operated to produce a gastric fistula. Recordings of changes in gastric tonus and motility were made by means of an intragastric balloon connected to a water manometer registering on a kymograph. This technique has been described by Carlson (5).

Following recovery from the operation, hypermotility was induced by subcutaneous injection of 30 units of insulin (Lilly) given after a fast period of 24 to 30 hours.

Having established the type of response of the dog's stomach to insulin hypoglycemia, atropine sulphate in a dosage of 0.5 mgm. was given subcutaneously, and the effect recorded.

The experiments were then repeated and blood was withdrawn from the saphenous vein for sugar analysis, *a*, before insulin was given; *b*, during the period of induced hypermotility, and *c*, at the time the atropine effect was noted.

At the completion of the experiments with atropine, a series of tracings was made, using adrenaline hydrochloride, 1 to 1,000 solution. Three-tenths to 0.5 cc. of solution was given intravenously during the period of hypermotility. Blood was also withdrawn for analysis as when atropine was used. Blood sugar analyses were made according to the method described by Folin and Wu (6).

RESULTS. Twenty to forty minutes following the subcutaneous administration of 20 to 30 units of insulin (Lilly), there was a rise in gastric tonus of about 2 to 3 cm., accompanied by augmentation of rhythmic gastric contractions. This period of activity continued for from 30 to 75 minutes, ending with diminishing activity and lowering of tonus. Ten to fifteen minutes after the subcutaneous injection of 0.5 mgm. atropine sulphate given during a period of hyperactivity, marked inhibition to complete cessation of gastric movements occurred. At the same time, there was a fall of tonus, in most instances of 1 to 3 cm. See figure 1.

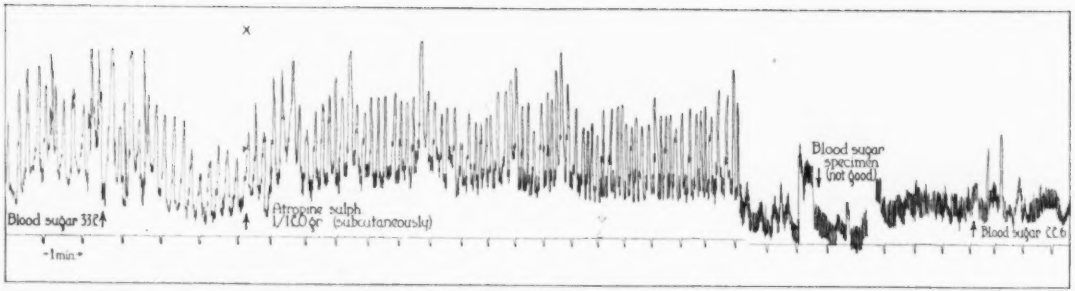

Fig. 1. This shows the hypermotility of the stomach on an increased tonus about 40 minutes after 20 u. insulin. At x (0.5 mgm.) atropine sulphate in aqueous solution was given subcutaneously. Seventeen minutes later the typical response to atropine is shown. Time interval, 1 minute.

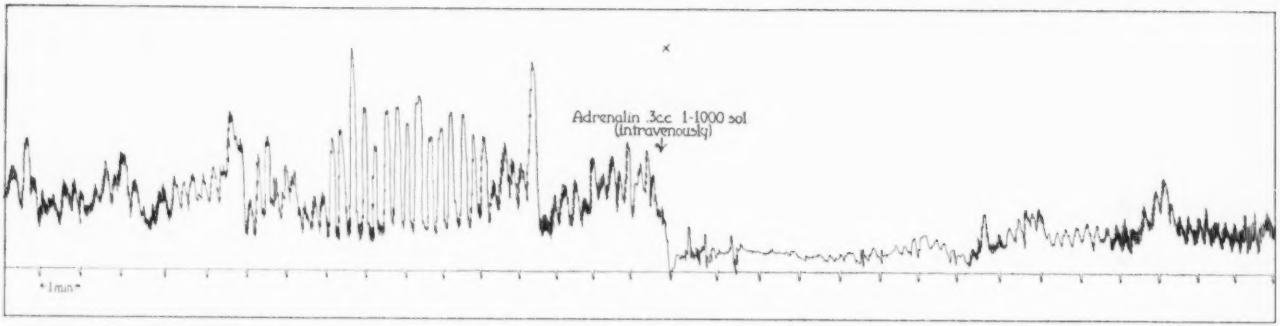

Fig. 2. The increased gastric contractions about 45 minutes after 20 u. insulin are shown. At x 0.3 cc. adrenalin chloride 1–1000 sol. was given. The inhibitory effect and lowered tonus are shown. Time interval, 1 minute.

Blood sugar readings on the fasting (24 to 36 hours) stomach showed readings around 0.075 to 0.085 per cent. Determinations made at the time hypermotility after insulin was established showed a fall in blood sugar values to around 0.025 to 0.035 per cent. Blood samples taken at the time of inhibition of contractions and fall of tonus following administration of atropine showed a further lowering of the blood sugar. See table 1.

When adrenaline 0.3 to 0.5 cc. of 1 to 1,000 solution was given intravenously during the period of hypermotility induced by insulin, an immediate response was recorded. See figure 2. The gastric tonus dropped 1 to 3 cm. with inhibition or complete cessation of the movements of the stomach. Blood sugar readings made during the course of these experiments with adrenaline showed changes similar to those obtained when atropine was given. There was no increase in the blood sugar after adre-

TABLE 1

Blood sugar readings

BEFORE INSULIN	DURING HYPER MOTILITY AFTER INSULIN	DURING ATROPINE INHIBITION	DURING ADRENALINE INHIBITION
per cent	*per cent*	*per cent*	*per cent*
0.074	0.033	0.022	
0.062	0.043	0.033	
0.082	0.035		
0.105	0.057		0.046
0.086	0.037		0.020

naline at the time the inhibitory action of the drug was noted. In fact, there was a further slight decrease. See table 1.

Discussion. While the present work was being carried on Quigley (7), working along the same lines but using human subjects, reported the effects of atropine and adrenaline on the hyperactive stomach of man in a state of hypoglycemia. Our findings on the dog correspond with those which he has reported for man.

From the results obtained in our work, which supports the report of Quigley, it seems probable that the increased tonus and hypermotility of the stomach which takes place when the blood sugar is markedly lowered following insulin, is due in part at least to increased irritability of the vagus motor nerve fibers to the stomach. It is further shown that this activity can be inhibited by stimulation of the splanchnic nerves, using adrenaline. Quigley (7) suggests that the inhibition following adrenaline may be the result of an increase in blood sugar, splanchnic stimulation, or both. We find no increase in the blood sugar after adrenaline at the time the effects of adrenaline are noted. There is, rather, a further slight lowering of the

sugar level. It is our opinion, therefore, that the immediate action of adrenaline given intravenously on the tonus and motility of the active stomach in hypoglycemia is the result of splanchnic stimulation only.

An increase in vagus irritability resulting from hypoglycemia which seems indicated by these experiments and which has been shown by others, might logically indicate a general increase in central nervous irritability which would account for the petulance, crossness and general emotional irritability of undernourished, hungry children, and also might explain the marked general increase in tonus of the starving or markedly hungry infant.

SUMMARY

1. It is shown that hypermotility produced in the fasting dog's stomach by the subcutaneous injection of insulin is inhibited, and the increased tonus is lowered by giving atropine in physiologic dosage. This effect is not accompanied by any increase in the blood sugar.

2. Adrenaline hydrochloride injected intravenously during gastric hypermotility accompanying hypoglycemia, caused a lowering of tonus and inhibition of the active stomach contractions. This effect was not due to increase in blood sugar.

3. The experimental results would seem to suggest the possibility that the hypertonicity of the starving infant may be due to an increased central nervous irritability caused to some extent by an existing hypoglycemia.

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NERVOUS CONTROL OF RESPIRATION

II. Types of Periodic Breathing Observed as a Result of Placing Lesions in the Brain-Stem

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The fact that respiration may be altered when the brain stem is sectioned caudal to the middle of the superior colliculi has long been known. It was noted in some cases by the earlier workers (reviewed by Bechterew, 1, Nikolaides, 2) that the slowing of respiration was due to a greater increase in the length of the inspiratory phase of each movement, while in other cases it was the expiratory phase which was more markedly increased. It was observed also that the effects of section of the mid-brain were more marked after section of the vagi. The prolongation of the inspiratory and expiratory phases by such sections were thought to be due to the elimination of *inhibitory inspiratory* and *expiratory centers* located in the stem at the levels of the inferior and superior colliculi respectively (2). Trevan (3) and Lumsden (4) have recently demonstrated that the sensitive area for ordinary respiration is caudal to the cephalic level of the pons. Lumsden noted consistently a very characteristic type of respiration after the cephalic pons had been injured. To the prolonged maintained inspiratory phase he gave the name "apneusis," which appears to be identical with Markwald's (5) "inspiratory cramp." From Lumsden's papers and textbook references it seems that he independently repostulated the idea of an inhibitory inspiratory center in the cephalic stem. And further, although he very correctly begins his paper by calling attention to the dangers of drawing conclusions from negative findings, he states definitely that in the cat, normal coördinated respiration is not possible unless the cephalic pons is intact, irrespective of the presence or absence of the vagi, and he does not mention the positive findings of earlier workers (Bechterew, 1) who state that an animal with the brain-stem sectioned as far caudal as the acoustic striae (intact vagi), respires normally and adequately enough to maintain the preparations for hours.

While investigating the possible course taken by the respiratory tracts in passing to and from the cephalic stem, certain definite types of periodic breathing have been observed in acute preparations under amytal anes-

thesia. Furthermore, observations have been made which appear to explain to some extent the mechanism of the periodicity.

RESULTS. *Inspiratory arrest.* The first type of periodic breathing observed was in the case of cat 101. Lateral lesions were placed simultaneously at the middle level of the pons (see plate II). After a short period of artificial respiration, active periodic respiration set in. The periodicity consisted in *a period of respiratory stand-still in the inspiratory*

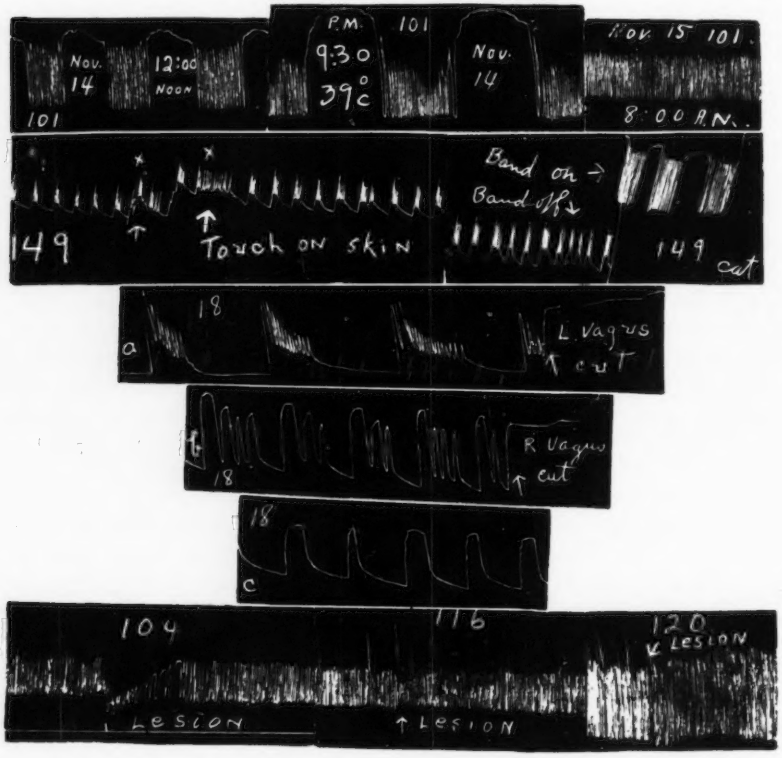

Plate I. Inspiration upstroke. Drum speed constant during each experiment

phase followed by a period of regular respiratory movements (see plate I). Touching the mucosa of the pharynx readily inhibited the inspiratory stand-still. This sequence of events continued for 10 hours at which time the respiratory recording apparatus was taken off and the animal was placed in an incubator. The following morning the animal's respiration was normal even when the usual respiration recording band was used. The type of periodic breathing above described has also been observed in

intact preparations under deep amytal during central sciatic and skin cutting stimulation.

Expiratory arrest. This type of periodic breathing was first met as a result of placing lateral lesions in cat 18 (see plate II). The second lateral lesion eliminated respiration entirely. Artificial respiration was given. Contact of the hand on the chest to feel the heart elicited active inspiration, the respiratory muscles being in the passive expiratory state. After

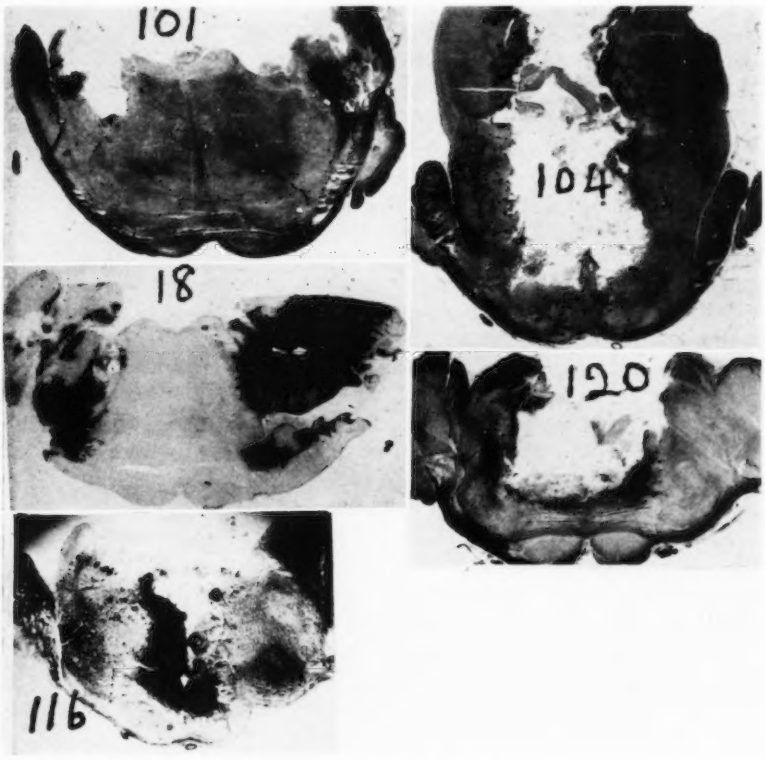

Plate II. Lesion photographs

a short interval, periodic breathing continued after cessation of artificial respiration. In this case the periodicity consisted in *a period of respiratory stand-still in the expiratory phase followed by a period of regular respiratory movements* (see plate I). This type of periodicity continued for three hours and at that time the left vagus nerve was cut. Temporary inspiratory arrest came on followed by the periodic breathing above described; however, the period of expiratory arrest was now of shorter duration and the

respiration rate slower (see b, cat 18, plate I). A few minutes later the right vagus was cut. There was a temporary inspiratory arrest followed by regular respiratory movements at the rate of three per minute (see c, cat 18, plate I). Respiration at this rate continued for somewhat more than half an hour when death occurred from respiratory failure. Thus, all signs of periodicity disappeared and the usual respiratory picture after brain-stem section-vagi section supervened.

Change in type of periodicity. In a preparation with the stem transected through the upper medulla (caudal end of cerebellar peduncles dorsally, and caudal pons ventrally) the inspiratory arrest type of periodic breathing occurred after a short interval of artificial respiration. Immediately upon removing the recording band from around the abdominal wall the *expiratory arrest type* of periodic breathing replaced the former. On reapplying the band the inspiratory type was resumed (cat 149, plate I). Since it was first observed, the change of the type of periodicity by removing and reapplying the recording abdominal band has been readily demonstrated on all preparations with bilateral lesions or with complete transections through pons or upper medulla. In this case as in all other experiments, touching the mucosa of the pharynx invariably inhibited the maintained inspiratory arrest, while touching the skin of the animal especially in the region of the thorax elicited active inspiration during expiratory arrest (see cat 149, plate I).

In these preparations, if artificial respiration is not given or active respiration induced by applying the proper stimulus (pharynx or skin), the animal dies without any attempt at breathing. The heart continues for from 2 to 3 minutes before its failure sets in.

Localization of the elements concerned in pons and upper medulla. Pontile preparations (section between inferior and superior colliculi dorsally and cephalic level of pons ventrally) can be prepared without any signs of periodic breathing. In these animals respiration may not be interfered with at all by the section. In some instances normal respiration is maintained for from one to three hours after transection at which time respiratory failure may occur without any warning. The last few respiratory movements become progressively shallower and terminate with cessation of all respiratory movements. This same type of respiratory failure has been observed after wide medial transverse lesions at pontile and upper medullary levels. (See cats 104 and 120, plates I and II.) In one experiment in which the level of section passed through the cephalic level of the mid-brain, a short time after the section was placed, periodic breathing of the inspiratory type began. This gradually gave way to the expiratory type. Then very suddenly normal regular respiration again set in and was maintained. The periodic breathing lasted only for an interval of five to six minutes.

A unilateral lesion or hemisection of the stem at a pontile or upper medullary level under amytal anesthesia results immediately in a marked slowing of the respiratory rate. A typical record of this is seen in plate I, cat 120. After such a lesion the reduced respiratory rate usually persists for several hours and then gradually returns to normal. In one case the normal rate was resumed an hour after hemisection of the stem through the pons. In a few cases animals were lost after a unilateral lesion from respiratory failure (maintained inspiratory position) before it was learned that removing the recording band immediately allowed for inhibition of the maintained inspiratory state.

Medial transverse lesions through the caudal pons and upper medulla that leave the extreme lateral regions of the stem intact affect respiration only temporarily (see cat 116, plates I and II), the effect usually being acceleration followed by a gradual slowing. However, the normal rate is resumed within a few minutes after the section. In placing such a lesion one can judge by the respiratory effect (time taken for return to normal) with a fair degree of accuracy whether or not the lesion has involved the medial stems only or has passed laterally so as to involve the lateral stem on one side (see cat 120, plates I and II).

Complete lower transection without periodic breathing. The fact that *normal uninterrupted* as well as *rapid breathing* is still possible after

Cat 65. Experiment December, 1928

TIME	RESPIRATORY RATE	RECTAL TEMPERATURE
		°C.
11:08 skull intact	230	36
12:00 lesion	150 apnea	36.5
12:01	15	
12:03	22	
12:07	26	
12:18	36	
12:25 Treachea cleaned	Strong coughing	
12:27	180	37
1:02	125	
1:08 after trachea cleaned	160	
1:12	108	37.2
1:30	50	37.7
1:53	50	38
2:15 after trachea cleaned	90	38.1
3:20	25	
3:21 extreme coughing elicited by attempt to expose femoral vein which resulted in respiratory failure		

complete stem section below the cephalic pons level is indicated in experiment 65. This cat was under light urethane anesthesia and the respiratory record was taken from the side tube of a trachea cannula. The stem was completely sectioned through the cerebello-pontile peduncles dorsally and the caudal tip of the pons ventrally. Placing a band around the abdomen for purposes of recording respiration immediately caused respiratory standstill in the inspiratory position. A protocol of the experiment is shown on previous page.

It is possible that the hyperpnea was of vagal origin since it occurred as a result of mechanical cleaning of the trachea. The preparation was under extremely light anesthesia with greatly increased reflex excitability.

Discussion. Markwald's description of "inspiratory cramp" and Lumsden's description of "apneusis" are practically identical. It seems probable therefore that they observed the same type of alteration of respiration following pontile transections. The type of periodic breathing characterized by inspiratory arrest described in this paper differs from these in one essential respect. Whereas Markwald and Lumsden speak of the respiratory movements occurring between the periods of inspiratory standstill as being convulsive and abnormal, in these cases, as can be seen from the tracings (see plate I), the movements are perfectly complete and normal.

In so far as can be determined the expiratory type of periodic breathing has not been previously described. It is, however, highly probable that the literature on this subject has not been completely covered.

There can be no doubt that the genesis of the periodicity in these cases is in part of peripheral origin. The prolonged inspiratory arrest is the result, under experimental conditions reported, of stimuli from cutaneous receptors. This is shown not only by the appearance of this type of periodicity when continued pressure is applied on skin by the recording band, stimulation of central end of sciatic and skin cutting under deep anesthesia, but also by the fact that active inspiration is readily induced by pressure, usually light pressure, on the skin during expiratory arrest. The inspiratory arrest seems to originate from the respiratory tract and lungs, since vagal section progressively decreases and eliminates the periodicity. Likewise the inspiratory stand-still is readily inhibited by mechanical stimulation of the pharyngeal mucosa. This does not necessarily imply, however, that such impulses can only arise from the above cited areas. Other undeterminated factors must of necessity enter in as shown by the fact that inspiratory stand-still can, in some cases, be inhibited by stimulation of the central end of the sciatic nerve.

There is no doubt, when the brain-stem is sectioned caudal to the cephalic level of the pons, that the *central mechanism for ordinary respiration under conditions of surgical anesthesia*, is markedly deranged. Whether the de-

rangement is due to an actual destruction or elimination of integrating pontile centers or simply to the partial derangement of lower bulbar centers is of course still a matter of conjecture. It is perfectly safe, however, to state that present evidence strongly favors the presence of pontile centers. It is readily seen from the foregoing results that if pontile centers are concerned they are located in the extreme lateral regions of the brain-stem and likewise that the tracts going to and from these centers course laterally through the pons and upper medulla. If bulbar centers are alone concerned the elements responsible for the derangement are located in the lateral regions of the pons and upper medulla.

Henderson and Sweet (6) have recently suggested that the mechanism of "inspiratory cramp" or "apneusis" is simply rigidity of the inspiratory muscles resulting from cutting the rubro-spinal tracts. That such is not the case is seen from the following facts: first, inspiratory arrest occurs under surgical anesthesia (complete atonia of striated muscle): secondly, the rubro-spinal tracts can be sectioned either by a mid-brain and upper pons longitudinal mid-line lesion or by transection of the stem at the caudal limits of the red nuclei, without any alteration of respiration.

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STUDIES ON DIABETES INSIPIDUS. IV[1]

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A review of the literature (Geiling, 1926; Fink, 1928; Futcher, 1929) indicates that the question of the relationship of the tuber cinereum and the hypophysis to diabetes insipidus will not be settled easily by a study of localized lesion of the hypothalamus or by simple hypophysectomy. The development of diabetes insipidus following injury to the tuber cinereum without apparent injury to the hypophysis may be due to the disturbance of the tuber cinereum per se, or to interference with the formation or discharge of the antidiuretic hormone of the hypophysis through nerve connections between the two, or to destruction of hypothetical centers controlling salt and water metabolism normally sensitized by, and therefore dependent upon, the antidiuretic hormone of the hypophysis. On the other hand, if diabetes insipidus is caused by lack of the antidiuretic hormone of the hypophysis, hypophysectomy might fail to produce it if sufficient quantities of the hormone can be secreted by the compensatory activity of portions of pars tuberalis left attached to the tuber cinereum (Towne, 1929), or of the tuber cinereum proper (Sato, 1928; Trendelenburg, 1928).

Total destruction of both hypophysis and hypothalamus should determine whether or not diabetes insipidus is a deficiency phenomenon. The production of diabetes insipidus by injury to the hypothalamus following transection of the brain stem caudad to the mammillary bodies should throw light on the part played by hypothetical centers. This paper reports the results of such experiments.

METHODS. *Hypophysectomy and destruction of the hypothalamus. Acute experiments, dogs.* Dogs were used, which had been fed 12 hours previously and allowed water ad libitum. The operation was performed by oral approach under ether anesthesia. Destruction was effected by electric cautery, an effort being made to destroy everything in the Circle of Willis to a depth of 3 to 4 mm. Following cauterization the area was swabbed with cotton to remove all débris. The entire operation required from 20 to 30 minutes and entailed little hemorrhage.

[1] Aided by a grant from the Committee on Scientific Research of the American Medical Association and by a fund established by the Ciba Company of New York.

Ether anesthesia was discontinued promptly. The bladder was emptied by catheter at intervals of half an hour throughout the experimental period, record being kept of urine volume and reducing properties, condition of the reflexes, of rate and character of respiration, of the filling of the veins on application of a tourniquet, of rectal temperature, and general activity. Judged by these criteria animals, which had been operated as described, are in perfect condition for experiments on diuresis for periods of 20 to 72 hours or more.

The destruction produced in these experiments was so extensive (figs. 1 and 3) that histological examination of the brain was not made.

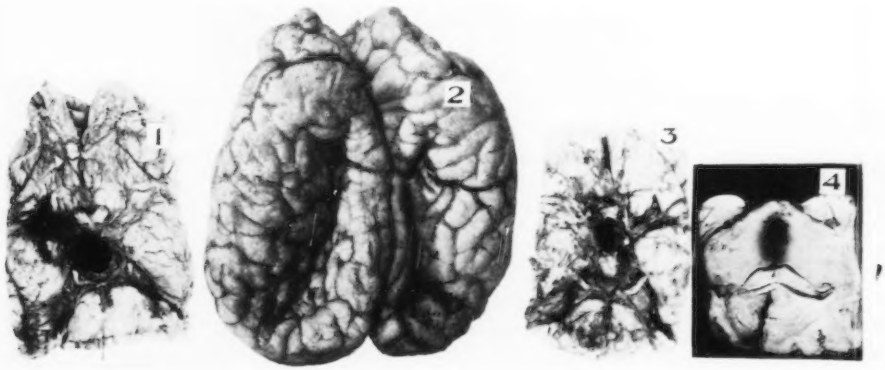

Fig. 1. Brain of dog 22, table 2
Fig. 2. Brain of dog 5, table 1
Fig. 3. Brain of dog 3, table 1
Fig. 4. Section through mammillary bodies of the brain of dog 3, table 1

Acute experiments, rabbits. Destruction of the hypophysis and hypothalamus was effected by transection of the brain stem caudad to the mammillary bodies and removal of everything rostral to the cut. After discontinuing the ether, paraldehyde was injected intraperitoneally as needed to control movements of progression. Rabbits secrete a normal volume of urine often heavily clouded with oxalates and respond actively to diuretics for periods of 10 to 60 hours following this operation.

Survival experiments, dogs. The operation was performed by the oral route, in which case destruction was produced by electric cautery, or by the temporal route, in which case various methods were used to produce destruction. For the first 14 to 20 hours following the operation no record was kept of small increases in urine volume output. Beginning with the 14th to 20th hour accurate records of urine volume and reducing properties

and of general condition were kept for one week following the operation and at intervals thereafter for a period of 40 to 150 days.

Each brain was examined histologically to define the extent of the destruction and marked histological change which had been produced. The more detailed nuclear changes were not studied.

Transection of the brain stem with production of diabetes insipidus. Pigeons were used. Following extirpation of the cerebral hemispheres, the brain stem was transected on a plane passing from the rostral extreme of the cerebellum to a point caudad to the oculomotor nerves by means of a blunt metal plate, thus leaving the blood supply to the hypophysis and hypothalamus intact. The hypothalamus was then injured on the dorsal side by means of an electric cautery (Rogers, 1923, 1924); the brain cavity was loosely packed with cotton; and the bird placed in an incubator. The experiments were controlled by transecting the brain stem of a second group of pigeons without further injury, and by cauterization of the hypothalamus of a third group without transecting the brain stem.

Results and discussion. *Hypophysectomy and destruction of the hypothalamus. Acute experiments, dogs.* Forty experiments are reported which fall into 3 groups—a group in which urine volume was normal throughout the period of observation, a group in which glucosuria but no diabetes insipidus developed, and a group in which diabetes insipidus did develop.

Group 1, urine volume normal. This result was obtained in 15 experiments. The average duration of 14 of the experiments was 9.2 hours. One experiment was continued over a period of 24.5 hours. Experiment 22, table 2, is characteristic of the results obtained.

Figure 1 gives a typical picture of the destruction produced in 14 of the experiments. It will be noted that essentially everything in the Circle of Willis as far caudad as the region of the oculomotor nerves was destroyed including the hypophysis, hypothalamus, and walls of the third ventricle. In the last experiment the destruction was less complete but included the infundibulum, tuber cinereum, and walls of the third ventricle. The posterior lobe of the hypophysis was adhering to the sella turcica.

If diabetes insipidus is a deficiency phenomenon, it would be expected to develop in these experiments. The brief period of experimental observation would not seem to account for its failure to occur. Verney, 1926, found that the effective quantities of the antidiuretic hormone of the hypophysis disappeared from his perfusion mixtures in 30 to 60 minutes and that the deficiency could be remedied by introducing a surviving head into the perfusion circuit but not by introducing a surviving head after hypophysectomy or by introducing a surviving leg. It is well known that the pressor effect of pituitrin is transient. It is quite possible therefore that the secretion of the hypophysis disappears from the intact animal fairly rapidly. Furthermore the latent period of the development

of diabetes insipidus in the majority of the experiments in group 3 of this series fell well within the period of observation in these experiments. The condition of the animal might be a factor though it responded to diuretics in each instance, there were no symptoms of failing circulation or respiration, and the results obtained in group 2 and on dog 2 of the survival experiments make it improbable.

Group 2. Glucosuria, no diabetes insipidus. This result was obtained in 13 experiments. The period of observation lasted for from 8 to 14 hours in 9 experiments, averaging 10.85 hours. The glucosuria developed promptly in 7 experiments, and after latent periods of 2 and 2.5 hours in 2. It persisted throughout the entire experimental period in 5 cases and terminated in 2.5 to 9 hours in the other 4. Experiment 3, table 2, is typical.

The period of observation in the remaining 4 experiments of this group was continued for 25, 26, 60.5, and 62 hours. The protocol for experiment 32 is characteristic of the results obtained:

8:00 a.m. Operation completed. (Weight of dog, 6 kilos.)
9:55 a.m. Glucosuria appeared and persisted 10 hours. Urine volume 8.7 to 11.6 cc. per hour.
9:00 p.m. Urine non-reducing. Volume normal. Dog ignores water; 200 cc. given by stomach tube.
7:30 a.m. 163 cubic centimeters of non-reducing urine. Dog ate voluntarily. For next 37 hours milk and water ad libitum: 257 cc. of non-reducing urine excreted.

In 10 of these experiments including number 32 and experiment 3, table 2, destruction in the Circle of Willis was complete as far caudad as the caudal tips of the mammillary bodies or as the posterior perforated substance. In 2 experiments the posterior perforated substance was also destroyed. In the last experiment the mammillary bodies escaped destruction and a fragment of the posterior lobe of the hypophysis was adherent to the sella turcica.

Diabetes insipidus should occur in these experiments if it is due to a deficiency phenomenon. As in the case of group 1 not only is the period of experimentation hours longer than the average latent period of development of diabetes insipidus in the experiments of group 3 but there is an active glocosuria, which makes it unlikely that the condition of the animal is a limiting factor. This is confirmed by the results obtained on dog 2 described under the survival experiments.

Group 3. Diabetes insipidus. Diabetes insipidus developed in 12 experiments. Experiments 3, 5, and 6, table 1, and experiment 1, table 2, illustrate the type of results obtained. The latent period averaged 2.7 hours in 10 of these experiments. It was 12 and 18 hours in the other 2. The diuresis was characterized by sudden onset, relatively great intensity,

TABLE 1

EXPERIMENT	WEIGHT	TIME	URINE			RECTAL TEMPERATURE	FILLING OF VEIN	REFLEXES	RESPIRATION	STATE OF CONSCIOUSNESS	REMARKS
			Cubic centimeters	Cubic centimeters per hour	Reducing properties						
	kgm.					°C.					
3	8.6	9:40									Operation begun
		10:00				38.5	++	+++	+++	Coma	Operation complete. Catheterization. Ephedrine. Sulphate, 15 mgm.
		:35	11.0	18.8	++	38.5	++	+++	+++	Coma	
		11:35	23.0	23.0	+	37.0	++	+++	+++	Coma	
		12:35	49.5	49.5	−	37.0	++	+++	+++	Coma	
		2:20	120.5	62.8	−	36.5	++	+++	*	Coma	*Cheyne-Stokes
		3:20	162.0	162.0	−	36.5	+	+++	*	Coma	
		4:20	170.0	170.0	−	36.0	−	+++	*	Coma	Sacrificed—See figures 3 and 4
5	12.7	7:40									Operation begun
		8:15				39.0	+++	+++	+++	Apathy	Operation complete
		8:30									Catheterization
		9:20	8.0	9.6	+	39.0	+++	+++	+++	Apathy	
		11:10	110.0	60.0	−	40.0	+++	+++	+++	Apathy	*Hyperpnea; emesis; muscular twitching; slow pulse
		12:05	95.0	103.6	−	40.0	+++	+++	+++	Apathy	
		1:20	70.0	56.0	−	41.5	+++	+++	+++	Restless	
		2:00	2.0	3.0	−	42.0	++	+++	*	Coma	Sacrificed—See figure 2
6	15.9	8:00									Operation begun
		8:30				37.0	+++	+++	+++	Apathy	Operation complete. Catheterized

		9:30	6.0	6.0	−	37.0	+++	+++	+++	Restless	Paraldehyde, 5 cc.
		10:30	16.0	16.0	++	37.0	+++	+++	+++	Hypnosis	
		11:00	20.0	40.0	−	37.0	+++	+++	+++	Hypnosis	
		12:00	174.0	174.0	−	37.0	+++	+++	+++	Restless	Paraldehyde, 5 cc.
		1:00	320.0	320.0	−	37.0	+++	+++	+++	Hypnosis	
		2:00	180.0	180.0	−	37.0	+++	+++	+++	Hypnosis	
		3:00	190.0	190.0	−	37.0	+++	+++	+++	Hypnosis	
		4:00	12.0	12.0	+++	37.0	+++	+++	+++	Hypnosis	Sacrificed

TABLE 2

EXPERIMENT	WEIGHT	TIME	URINE			TEMPERATURE	FILLING OF VEIN	REFLEXES	RESPIRATION	STATE OF CONSCIOUSNESS	REMARKS
			Cubic centimeters	Cubic centimeters per hour	Reducing properties						
	kgm.					*°C.*					
1	8.6	8:55									Operation begun
		9:20				39.0	+++	+++	+++		Operation complete, catheterization. Paraldehyde—6 cc.
		10:45	9.5	6.7	++	39.0	+++	+++	+++	Hypnosis	
		2:10	46.0	13.2	+	38.5	+++	+++	+++	Hypnosis	
		4:05	100.0	52.2	−	38.5	+++	+++	+++	Hypnosis	
		4:50	70.0	93.3	−	38.5	+++	+++	+++	Apathy	
		5:50	93.0	93.0	−	39.0	+++	+++	+++	Apathy	Ignores water, drinks milk avidly,
		7:35	210.0	120.0	−	39.5	+++	+++	+++	Apathy	during night water allowed ad libitum
		8:35	2,525.0	194.2	−	?	+++	+++	+++	Normal	Sacrificed
22	8.5	8:45									Operation begun
		9:15				35.0	+++	+++	+++		Operation complete, catheterization
		10:15	3.4	3.4	−	35.0	+++	+++	+++	Restless	Paraldehyde, 5 cc.
		12:25	9.1	4.2	−	36.5	+++	+++	+++	Hypnosis	
		2:45	8.5	3.74	−	36.5	+++	+++	+++	Hypnosis	Ephedrine sulphate, 15 mgm.
		4:45	8.5	4.25	−	37.0	+++	+++	+++	Hypnosis	8 cc. Na_2SO_4 10 per cent sol., intra-
		5:25	13.0	19.5	−	37.0	+++	+++	+++	Apathy	venously
		5:55	5.0	10.0	−	37.0	+++	+++	+++	Apathy	

		7:00	8.5	7.8	−	37.6	+++	+++	+++	Apathy	
		8:00	4.5	4.5	−	38.0	+++	+++	+++	Apathy	
		8:30	2.1	4.2	−	38.0	+++	+++	+++	Apathy	Sacrificed—See figure 1
3	7.4	8:55									Operation begun
		9:20				36.5	+++	+++	+++		Operation complete
		9:50				36.5	+++	+++	+++	Coma	Ephedrine sulphate, 15 mgm. Catheterization
		10:50	18.0	18.0	+++	36.5	+++	+++	+++	Apathy	
		12:35	21.0	12.0	+++	37.2	+++	+++	+++	Apathy	
		3:50	25.0	7.5	+++	37.2	+++	+++	+++	Apathy	
		7:05	26.6	8.44	+++	40.5	+++	+++	+++	Apathy	
		8:10	9.0	8.3	+++	40.5	+++	+++	+++	Apathy	Sacrificed

and abrupt cessation. The duration varied markedly. The average duration in 8 experiments was 3.8 hours. In 2 experiments it lasted 11 and 17 hours. The animal was sacrificed while still diabetic in the other 2 after periods of 5 and 6 hours of diuresis.

Experiment 5, table 1, of this group is of interest in that the diabetes insipidus ceased abruptly and symptoms of hemorrhage into the fourth ventricle developed. The dog was sacrificed and the hemispheres were found collapsed and very thin suggesting that in this experiment the brain had been desiccated (fig. 2). There was no evidence of hemorrhage. Experiment 1, table 2, throws light on the phenomenon of thirst since after 5 hours of diuresis the dog repeatedly ignored water but drank milk hungrily. It is also of interest that the diabetes insipidus was preceded by glucosuria in 10 of the experiments and that glucosuria reappeared after it had terminated in 3 of them.

Destruction was uniformly less extensive in these experiments than in those of groups 1 and 2 in which diabetes insipidus failed to develop. In 4 experiments the posterior lobe of the hypophysis was found adherent to the sella turcica. In all of the experiments the tuber cinereum and adjacent walls of the third ventricle were destroyed but the mammillary bodies, though more or less injured, were present. Figures 3 and 4 were representative of the condition of the brains in this group of experiments.

Several facts brought out by these experiments indicate that diabetes insipidus is an irritation phenomenon dependent upon centers rostral to the posterior perforated substance. It failed to appear in 10 experiments in which everything in the Circle of Willis rostral to the posterior perforated substance or caudad tips of the mammillary bodies was destroyed, and in 16 experiments in which everything in the Circle of Willis rostral to the oculomotor nerves was destroyed (experiments, groups 1 and 2) but it did appear in 12 of 14 experiments in which the mammillary bodies escaped destruction. The latent period, the duration, and the intensity of diuresis are variable. It is typically transient. The onset and termination are more or less abrupt. These characteristics apply equally well to the diabetes insipidus produced in survival experiments in which water is allowed ad libitum. They indicate that the polyuria is due a diuretic substance or to the irritation of hypothetical centers controlling salt and water metabolism, a possibility which would seem to be eliminated by experiments to be discussed further over in this paper, however.

Evidence in the same direction has been obtained by Bayliss and Fee, 1930, who have found that kidneys in situ with nerves intact do not develop diuresis when perfused with a heart-lung preparation.

Acute experiments, rabbits. Thirty-eight experiments are reported. The results parallel those obtained in dogs in every respect. In 23 of the 26 experiments, in which the brain stem had been transected caudad to the

mammillary bodies, urine volume was within normal limits and the kidneys responded to diuretics during the period of observation averaging 10.3 hours. Glucosuria developed in the other 3. In 12 of 18 experiments, in which the transection was just rostral to, or through the rostral portion of the mammillary bodies, diabetes occurred.

Survival experiments, dogs. The animals reported were in good general physical condition at the time they were sacrificed as far as could be judged of dogs confined indoors under care. None of them manifested symptoms of adiposity or dystrophy, results confirming those obtained by Smith, 1927, on rats. As the dogs were not catheterized at the time of operation and the urine output during the first 14 to 20 hours was not noted unless it exceeded a volume of 800 cc., such transitory diabetes insipidus as occurred in some of the acute experiments (table 1, experiments 5 and 6) would have been overlooked in these experiments. The statement in the following paragraphs that diabetes insipidus did not occur means therefore that no diabetes insipidus was noted after the first 14 to 20 hours and that, if any occurred before that time, it was very transient or moderate in degree.

Thirty-six experiments are included. Three were diabetic at the end of the experimental period. The rest fall into three groups according to the type of lesion produced.

Injury was limited to partial destruction of the posterior lobe and infundibulum and to minor injury to the adjacent tuber cinereum in 14 experiments. Diabetes insipidus did not develop in any of them. Likewise it failed to develop in the 3 experiments in which the infundibulum was completely separated from the brain stem and the tuber cinereum destroyed, a condition which would be expected to produce diabetes insipidus if it is due to the lack of a hormone discharged into the ventricle by these parts. Dog 2 is of special interest in that the destruction was almost as extensive as that reported in the acute experiments on dogs, group 2. Hypophysectomy was complete, the hypothalamus was destroyed from the optic chiasma to the caudal extreme of the mammillary bodies and the walls of the third ventricle were partially destroyed. This dog was kept for 3.5 months after the operation. At the end of that period it seemed to be perfectly normal.

Transient diabetes insipidus developed in 5 of 16 experiments in which the injury involved the hypothalamus caudal to the infundibulum. No correlation between the site and extent of the lesion and the development, duration, or intensity of diabetes insipidus could be found. For instance, the brains of dogs 63 and 55 showed essentially the same lesion, that is, severe injury to infundibulum and adjacent tuber cinereum, destruction of the caudal tuber cinereum and the nuclei dorsal to it as far as the fornix, and destruction of the ventral half of the mammillary bodies. Number

63 was diabetic for a period of 2 weeks while number 55 failed to become diabetic.

In the three experiments in which the animals were still diabetic 3, 3.5, and 3 months after the operation, the histological picture differed in each. In dog 239, weighing 9 kilos and passing 1850 cc. of urine per 24 hours, the infundibulum and adjacent tuber cinereum were injured, one mammillary body was destroyed and the second injured along the medial surface. In dog 102, weighing 66 kilos and passing 750 cc. of urine per day, the infundibulum was injured and the tissue capping the ventral portion of the third ventricle was infected through the region of the caudal tuber cinereum and the mammillary bodies. In dog x, weight 11.2 kilos and passing 820 to 1000 cc. of urine per day, a line of central injury passing through from the ventral surface to the ventricle between the optic chiasma and posterior perforated substance partially destroyed the tuber cinereum, infundibulum, and the mammillary bodies.

These experiments confirm the results obtained in the acute experiments reported insofar as they parallel them. They indicate that some factor other than lack of a hormone is involved in the phenomenon of diabetes insipidus and that the nature of the disturbance produced rather than the site or extent of lesion determines its development in agreement with Hanchett, 1922.

Transection of the brain stem with development of diabetes insipidus. Experimental diabetes insipidus of average duration and intensity was produced in 13 pigeons in which the brain stem was completely transected, with injury to the hypothalamus, in 10 pigeons in which the transection was complete except for lateral strands of the optic lobes, and in 8 pigeons in which the hypothalamus was injured without transection of the brain stem. It failed to develop in 10 pigeons in which the brain stem was transected without injury to the hypothalamus.

These experiments demonstrate that in pigeons descending nerve tracts are not involved in the phenomenon of diabetes insipidus. The facts that diabetes insipidus in dogs is not stopped by high transection of the spinal cord or paralysis of the parasympathetic nervous system, that it can be induced after transection of the spinal cord (Bourquin, 1927) and after splanchnectomy and sympathectomy in dogs (Rubio, 1927) indicate that descending nerve tracts are not involved in this disturbance in dogs. The fact that transection of the brain stem of the rabbit caudal to the mammillary bodies does not produce it is partial proof in the same direction.

SUMMARY

1. The results of injury to the hypothalamus and hypophysis of dogs allowed to survive 40 to 150 days after operation are reported.

a. Symptoms of adiposity and dystrophy did not develop in any of these experiments, results confirming Smith, 1927, on rats.

b. In all of the experiments, in which diabetes insipidus developed, the lesion involved the caudal portion of the hypothalamus. There was no correlation between the site of lesion and the occurrence, intensity, or duration of the diabetes insipidus, however.

c. Diabetes insipidus failed to develop after separation of the infundibulum from the brain stem and destruction of the tuber cinereum in 2 experiments, and after destruction of the hypophysis and entire hypothalamus with the exception of the caudal tips of the mammillary bodies in one experiment.

d. These experiments indicate that diabetes insipidus is not a deficiency phenomenon and that the nature of the disturbance produced determines its development.

2. Diabetes insipidus did not develop after complete destruction of hypophysis and hypothalamus in 26 acute experiments on dogs and 26 acute experiments on rabbits. It followed destruction of the hypophysis and tuber cinereum accompanied by injury to the mammillary bodies in 12 of 14 acute experiments on dogs and 12 of 18 acute experiments on rabbits. These results indicate, a, that diabetes insipidus is not a deficiency phenomenon; b, that the mammillary bodies or centers in their vicinity are essential to the phenomenon, and, c, that the polyuria is caused by a diuretic substance or irritation to hypothetical centers controlling salt and water metabolism.

3. Diabetes insipidus was produced by injury to the hypothalamus in 23 pigeons after transection of the brain stem caudad to the oculomotor nerves in such a way as to leave the blood supply intact but did not develop after simple transection of the brain stem at the same level. These experiments demonstrate that in pigeons descending nerve tracts are not essential to the phenomenon of diabetes insipidus confirming similar experimental evidence in dogs (Bourquin, 1927) (Rubio, 1927).

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THE OXYGEN-CONSUMPTION OF ISOLATED MUSCLES FOR ISOTONIC AND ISOMETRIC TWITCHES

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Ever since myothermic methods have been used for investigation of the energy liberation of isolated muscles, the question whether more energy is liberated in isometric or in isotonic contractions has been of considerable interest. Heidenhain (1864), Fick (1878, 1884, 1885), Schenk (1892), Greife (1895) and Blix (1901) stated that an isometric contraction gave in general more heat than an isotonic. In particular Heidenhain and Fick found that in some cases an isotonic contraction under a heavy load gave distinctly more heat than an isometric contraction. Later on A. V. Hill (1913, 1914, 1916) concluded that on stimulation a muscle develops a given amount of heat and a given amount of elastic potential energy, of which a varying part can be recovered as work. Therefore an isotonic contraction never produces more, and under certain conditions produces less heat than an isometric, because the energy liberated is proportional to the average working length of the muscle fibers at the time of the energy liberation. About ten years later, Fenn (1923) re-investigated this question in Hill's laboratory and concluded that for single twitches, as well as for short tetani, there was a fairly good quantitative relation between the heat produced by the muscle and the work done. Fenn believed that the difference between his results and those of previous investigators was due to the fact that he used the sartorius muscle of the frog instead of the gastrocnemius. Without doubt a greater theoretical significance may be claimed for results obtained with sartorius muscles on account of their greater anatomical simplicity. The influence of the arrangement of fibers upon the relation between work performed and heat liberated was later demonstrated by Martin (1927) in Fenn's laboratory.

Some years ago I repeated experiments of this kind with sartorius muscles (Fischer, 1928), and obtained the same result as Fenn for short tetani. Using single induction shocks (stimulating directly or indirectly) I succeeded in getting a larger heat development for isotonic twitches than for isometric, but only when the temperature was lower than 17°C. At higher temperatures the isometric heat was always as large or larger than

the isotonic. A few months later Hartree and Hill (1928) published experiments from which they also concluded that after tetanic stimulation of a frog sartorius more energy can be liberated if work be performed than in an isometric contraction; but that in single twitches the total energy set free is the same whether work be done or not.[1] Their experiments were done at various temperatures and in all cases they compared the isometric twitch with *only one single* twitch performing work on the Levin-Wyman ergometer (1927); while in the experiments of Fenn and in my own the isometric twitch was always compared with a whole series of isotonic twitches of increasing load. Working with isolated mouse muscles (Fischer, 1930a) I concluded again that for short tetanic stimulations, isotonic contraction with a large load gives a greater heat production than isometric contraction. Unfortunately technical difficulties with the mouse muscle prevented the use of single shocks for stimulation.

The question of theoretical importance is, whether (for a single twitch at a given muscle length) one can establish an "energetic all-or-none theory" of muscular activity. The fact that most of the investigators mentioned above found for isotonic twitches with very small loads a much smaller heat production than that which would correspond to isometric twitches of the same average acting muscle length, is—(as A. V. Hill pointed out)—no proof against such an all-or-none theory. The shortening of a muscle with a very small load is so great that no one who has had experience with the myothermic method would deny the possibility that the low heat production of some isotonic twitches may be due largely to the slipping of the muscle over the junctions of the thermopile.

Under these circumstances one must try to determine the energy change of the muscle for both kinds of twitches by other methods. The few previous attempts which have been made to do this by chemical methods have been very contradictory. Riesser (1929) and co-workers (Nagaya, 1929) found a larger production of lactic acid for isotonic than for isometric twitches. Embden, Hefter and Lehnartz (1930) stated that the contractions appearing when muscles are immersed in liquid-air show an increased formation of phosphoric acid with increased load. Rothschild (1930) on the other hand found the lactic acid formation of sartorii independent of the load, while for gastrocnemii and semimembranosi the chemical change increased with increasing load. He found in no case a higher lactic acid formation for isotonic twitches than for isometric. But it seems that he did not compare isometric twitches with isotonic twitches under heavy loads.

[1] During the preparation of this paper, A. V. Hill published a new paper (Proc. Roy. Soc. London B. cvii, 115, 1930) describing recent myothermic experiments, in which he succeeded in finding the Fenn-effect for single twitches, and he now reaches conclusions similar to those drawn in this paper.

My own experiments described in this paper were intended to decide the question of the Fenn-effect by measuring the oxygen consumption of one muscle for several periods of work, using isometric twitches and isotonic twitches with different loads.

METHOD. The apparatus for the oxygen measurement was a Thunberg differential volumeter of the type generally used in the Rochester laboratory (Fenn, 1927a, b). Each of the two bottles held about 12 ccm., and a movement of 1 cm. of the kerosene drop in the capillary corresponded ordinarily to an oxygen consumption of 3.88 cmm., when the carbon dioxide liberated from the muscle was absorbed by sodium hydroxide.

In order to be able to vary the load on the muscle and to record the contraction outside the respirometer I used a "stuffing box" consisting of a glass capillary tube 5 mm. long and 0.3 mm. in diameter sealed in the stopper of the respirometer bottle. A wire 0.25 mm. in diameter passed through this capillary connecting the muscle to the lever. The narrow space around the wire was sealed with oil of medium consistency.

Meyerhof (1927) has criticized Parnas (1921) for the use of two such "stuffing boxes" in his apparatus, believing that it is likely to introduce errors. In my experiments it was impossible to avoid the use of one "stuffing box" but I tried to determine by control experiments the importance of any resulting error in the oxygen measurements. For this purpose I replaced the muscle inside the respirometer by a weight fixed to the wire. Then the wire which passed through the "stuffing box" was moved rhythmically and quickly to simulate as nearly as possible the movements which would be caused by a muscle contracting under a very small load. While the wire was being moved, the kerosene drop showed vibrations, but as soon as the movements were stopped at the original position of the wire, the drop returned exactly to its original position. A second test consisted in a short warming of one of the bottles, so that the drop moved very quickly in the capillary. When the temperature settled down again the drop went back exactly to its original position. During this test a much greater and quicker difference in the pressure inside and outside the bottle occurred than during an experiment with the muscle. These tests seem to me to afford sufficient proof that any error introduced by the stuffing box is negligible.

The muscles used for the chief experiments were always double sartorius preparations of *Rana pipiens*. In order to minimize the duration of the recovery period only small sartorius muscles 30 mm. in length were used. Meyerhof (1927) has already pointed out that in using direct stimulation of the muscle one cannot avoid an increased oxygen consumption due to injury, and as my own measurements of heat production (1928, 1930b) have shown an increased energy liberation by direct stimulation, I preferred to use stimulation through the nerve for all the experiments de-

scribed in this paper. For this purpose a pair of nerve electrodes was sealed into the stopper of the muscle bottle. The whole differential-volumeter was kept in a water-bath, the temperature of which was kept constant during each experiment to $\pm 0,01$°C. All experiments were performed between 11,5 to 12,5°C. The muscle contractions were registered on a slowly moving smoked drum.

RESULTS. There were two different types of experiments. In most experiments, after attaining a basal rate of oxygen consumption, the muscle was stimulated for one or two minutes with a series of single shocks (20 to

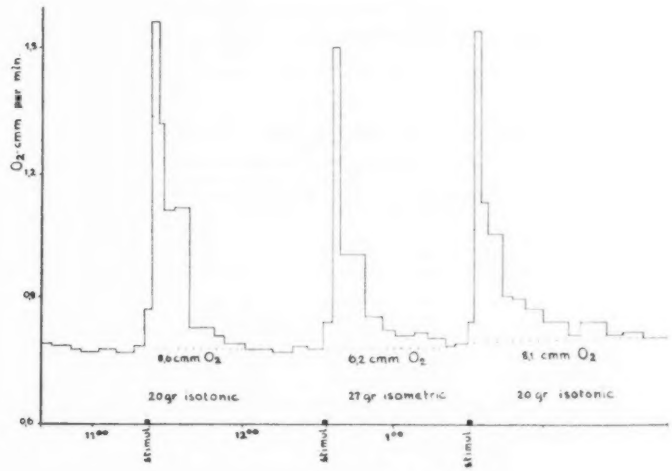

Fig. 1. Rates of oxygen consumption plotted as ordinates against times. Three series of 60 stimulations. The second series isometric, the first and the third isotonic against a load of 20 grams, shortening about 4 per cent of muscle length. Experiment of the 19.II.30. Temperature 12,0°C.

60). The excess oxygen consumption was measured until this basal rate was again reached (fig. 1). For the small muscles such as I used, this excess oxygen consumption is usually completed in 30 to 40 minutes. The data from three consecutive series of stimulations in such an experiment are plotted in figure 1. Control experiments without any change of the mechanical conditions showed that not only does the oxygen consumption remain constant as long as the tension developed or the work does not decrease, but that with decreasing mechanical response the oxygen consumption diminished in the same rate. The results of two of these controls are represented by tables 1 and 2.

In order to measure the oxygen consumption of the same muscle for different loads, I began the experiment with one series of isometric twitches. Then I decreased the load at each series until a load of 15 or 10 grams was

TABLE 1

Experiment of February 19, 1930. Isotonic load 20 grams

Each series consisted of 60 twitches

SERIAL NUMBER	WORK, GRAM CM. PER TWITCH	O_2-CONSUMPTION, GRAM CM. PER TWITCH	EFFICIENCY PER CENT
1	2,62	28,2	9,3
2	2,60	28,8	9,1
3	2,55	27,7	9,2
4	2,04	22,8	9,0
5	1,20	14,1	8,5

TABLE 2

Experiment of February 17, 1930. Isometric lever

Each series consisted of 40 twitches

SERIAL NUMBER	TENSION PER TWITCH IN GRAM	O_2-CONSUMPTION, GRAM CM. PER TWITCH	$\frac{O_2}{\text{TENSION}}$
1	65	71,0	1,09
2	64	71,6	1,12
3	64	70,2	1,09
4	63	71,4	1,13
5	50	55,6	1,15
6	41	48,3	1,17
7	28	34,6	1,23

TABLE 3

SERIAL NUMBER	LOAD, GRAM	SHORTENING IN MM.	WORK GRAM CM.	O_2-CONSUMPTION, GRAM CM.	EFFICIENCY PER CENT	$\frac{O_2}{\text{TENSION}}$
1	88	Isomet.		70,9	—	0,81
2	65	0,6	3,9	81,8	4,8	—
3	45	1,4	6,3	69,1	9,1	—
4	25	2,0	5,0	59,4	8,3	—
5	10	3,4	3,4	43,3	7,8	—
6	15	2,5	3,7	45,4	8,1	—
7	25	1,6	3,6	49,4	7,3	—
8	45	0,7	2,2	52,5	4,2	—
9	58	Isomet.		46,0	—	0,79

reached; then the load was increased again from series to series until the muscle worked isometrically. But as such an experiment lasted for six hours or more, the tension developed in the last series was much smaller than in the first one. Nevertheless the figures of table 3, which represent

such an experiment, show that the oxygen consumption for an isotonic twitch with a relatively heavy load is distinctly higher than for an isometric twitch. On the other hand a twitch with a very small load consumes much less oxygen than the isometric twitch.

It was characteristic of all these experiments that in isotonic twitches, where only a little shortening occurred, the oxygen consumption was higher than in isometric twitches, while for a large shortening much less O_2 was consumed. This relation seems to be valid for a fresh muscle as well as for a very fatigued one. It is therefore possible to calculate the average values for the oxygen needed at different loads, even though the working power of the muscle decreases during the course of the experiment. The average values were calculated always from those series of stimulations, which

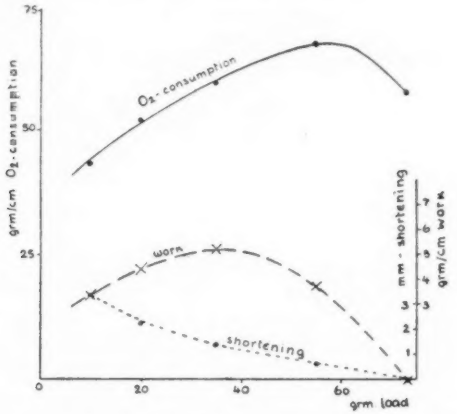

Fig. 2. The average values of oxygen consumption, work done and shortening plotted as ordinates against load. Experiment of the 13.III.30. Temperature 11,7°C.

showed approximately the same shortening of the muscle. Thus in the experiment of table 3, average values have been determined for both the isometric series, for the two series with a shortening of 0,6 to 0,7 mm., for the two series with 1,4 to 1,6 mm. shortening, and for the two series with 2,0 to 2,5 mm. shortening. The results are graphically represented by figure 2. The muscles of the other experiments showed the same behaviour.

In all experiments described above the excess oxygen consumption was measured for a certain number of twitches, occurring in a very short time. In order to further confirm my results I tried a new series of experiments in which the muscle was stimulated at a certain frequency (2 to 6 times per minute) throughout the whole course of the experiment; the resulting rate of

oxygen consumption in the steady state was then measured for different loads. Figure 3 represents the measurements of oxygen consumption in part of such an experiment. For this figure I have purposely chosen an experiment in which the muscle fatigued quickly, thereby demonstrating that typical variations in heat production with variations in shortening can be produced even with muscles in poor condition.

In table 4 are summarized all my experiments in which the muscle remained in a condition good enough to permit the calculation of average values. The amounts of oxygen needed for the different loads are arranged according to the amounts of shortening of the muscles. Only the one experiment (6.III.30) fails to show for an isotonic twitch with little shorten-

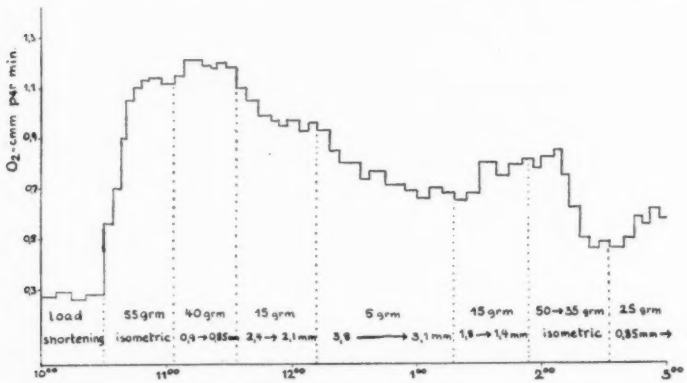

Fig. 3. Rates of oxygen consumption plotted as ordinates against times. Beginning at 10^{30} continuous stimulation at a frequency of 4 shocks per minute. Decrease and increase of the load at various times. Experiment of the 10.III.30. Temperature 11,7°C.

ing a higher energy-output than for the isometric twitch. Table 4 contains also all the values for the oxygen consumption (measured in grm./cm.) per gram tension developed and centimeter length of muscle. The average value for this isometric coefficient is

$$\frac{\text{grm.cm. oxygen}}{(\text{grams tension}) \times (\text{cm. muscle length})} = 0.380$$

Meyerhof und Schulz (1927) published only three single experiments with gastrocnemii where they found coefficients of 747, 755, 423 with an average value of 642. As Meyerhof's coefficient had the dimensions $\frac{(\text{kgm. tension}) \times (\text{cm. muscle length})}{\text{mgm. } O_2}$ it must be divided by a factor of

150.1 and inverted, to give a figure comparable with the isometric coefficient of my experiments. Thus $\frac{150.1}{642} = 0.234$.

If this figure is divided by 0.63 to take account of the diagonal arrangement of the fibres in the gastrocnemius (cf. Mashimo (1924) and Hill (1925, 1928a) then the figure obtained is 0.234/0.63 or 0.372 which is in striking agreement with my own figure of 0.380.

On the other hand one can compare this isometric coefficient with values of $\frac{H}{T \times L}$ calculated from the initial heat measured by the myothermic method. Hill (1928,b) found the ratio between the total heat in oxygen

TABLE 4

DATE (1930)	O_2-CONSUMPTION IN GRAM CM. FOR A SINGLE TWITCH					ISOMETRIC TENSION, GRAMS	$\frac{O_2}{T \times L}$	MAXIMAL EFFICIENCY PER CENT
	Shortening in per cent of muscle length							
	10	10 to 7	7 to 5	5 to 2	Isomet.			
February 16	—	16,1	—	19,2	17,2	14	0,385	7,5
February 19	—	—	—	30,1	24,4	32	0,428	8,0
February 21	—	—	22,2	33,8	24,9	37	0,480	12,0
February 27	17,0	18,4	—	25,8	19,1	18	0,354	7,6
February 28	—	—	—	18,3	17,5	15	0,488	4,1
March 3	16,3	18,5	21,5	20,2	19,9	19	0,425	13,8
March 5	—	15,2	—	21,8	20,2	18	0,325	9,3
March 6	—	64,0	—	64,7	66,0	45	0,505	7,3
March 8	23,6	34,6	—	40,4	35,9	47	0,240	11,5
March 10	23,8	—	34,6	35,1	30,2	45	0,258	8,2
March 13	43,3	52,2	59,3	67,1	58,4	73	0,281	9,1
March 14	—	18,7	—	21,9	20,0	18	0,371	10,3
March 16	—	68,3	—	89,5	76,5	82	0,301	8,6
March 19	—	43,1	—	50,8	45,7	42	0,374	9,3
March 21	—	—	25,2	27,1	24,5	18	0,425	6,8
March 24	24,7	26,3	—	30,2	28,5	21	0,453	7,2

and the initial heat for single twitches to be 2.07. Hence my value for $\frac{O_2}{T \times L} = 0.380$ corresponds to an isometric heat coefficient of $\frac{0.380}{2.07} = 1.84$. This value is in a fairly good agreement, as shown in table 5, with the values hitherto published for the isometric initial heat coefficient of sartorius muscles.

The last column of table 4 gives the maximal efficiency, which was observed during the course of each experiment, the average efficiency so found being 9 per cent. This value must be too small since optimal loads could not be found in every experiment. However the highest efficiency observed in any experiment was 13.8 per cent, and this in a good agreement

with the results of experiments by heat measurements. Fenn (1923) stated that 23 per cent was the maximal anaerobic efficiency for the frog sartorius. Hartree and Hill (1928) calculated it to be 26 per cent, while in my own heat experiments (1928) I found a maximal efficiency of 24 per cent. As 13.8 per cent anaerobic efficiency is equal to $13.8 \times 2.07 = 28.6$ per cent anaerobic efficiency, the conformity of the results described in this paper with the older experiments is very satisfactory.

I call special attention to this agreement, because this correspondence seems to me a proof for the exactness of both methods, the myothermic and the oxygen-consumption.

Discussion. From the results of this paper there can be no doubt that a single isotonic twitch against a heavy load, which allows the muscle only a small shortening, is accompanied by a larger energy change than an isometric twitch. A few experiments with tetanic stimulation gave the same result even when the shortening was relatively large. But without

TABLE 5

Comparison of isometric coefficients $\frac{H}{T \times L}$

VALUE $\frac{H}{T \times L}$	AUTHOR
0,220	Hartree and Hill (1921)
0,231	Fischer (1928)
0,163	Hill (1928b)
0,080	From Meyerhof's data
0,184	Present paper

doubt even for tetanic contraction the work done is not the only factor influencing the energy change of the muscle.

With single twitches, the amount of shortening occurring during the contraction is of much greater influence than the work done. By the shortening of the muscle the acting length of fibres is diminished. We know from experiments of Heidenhain (1864), reinvestigated by Hill (1925a) that the energy change of muscle (measured as initial heat production) depends upon the actual length of the fibres. But for twitches with only a small shortening, the decrease in energy liberation is smaller than the increase shown in the real Fenn-effect, if we call Fenn-effect the increasing influence of the work done upon the energy liberation.

From the experiments of A. V. Hill, mentioned above, one might argue that even the increased energy change observed for heavily loaded isotonic twitches may be due to the change in the fibre length. The heat production increases with increasing length of fibre only up to a certain point after which it decreases with futher extension of the muscle. There is therefore

an optimum length of muscle for isometric heat production which might be supposed to coincide with the average effective length of the muscle for heavy loads, at which a maximum heat production is also observed. But this objection is not valid, since in all the experiments of this paper as well as in my earlier myothermic experiments (also in those of Fenn) the resting length of the muscle was much smaller than the optimum.

A second question which must be mentioned here is: why is the Fenn-effect much more distinct for short tetani than for single twitches? One may suggest that the effect of fibre length may manifest itself in connection with the process of development of tension rather than with the maintenance of tension. For this reason it would have a greater effect in single twitches where all the energy is for development of tension and would thus serve to mask the true Fenn-effect, or the effect of work alone, on the energy production. If this suggestion is correct one must expect that with increasing duration of tetanic stimulation the excess energy change for work done becomes relatively greater. This was indeed the case in the heat experiments of Hartree and Hill (1928).

I take pleasure in expressing my deep indebtedness to the authorities of the University of Rochester for the invitation to work there as a visiting member of the Medical School, and for the great hospitality I enjoyed there. But particularly I owe thanks to Prof. W. O. Fenn, in whose laboratory these experiments were carried out.

SUMMARY

1. By means of a differential-volumeter, the increased oxygen consumption of a frog sartorius muscle after stimulation of the nerve by single induction shocks has been observed, for isometric twitches and for isotonic twitches of different loads.

2. Two types of experiments were performed; in the first, the excess oxygen consumption was determined for a series of 20 to 60 stimulations occurring in one or two minutes, while in the second type the equilibrium consumption was measured for continuous stimulation with a frequency of 2 to 6 per minute.

3. Isotonic twitches with large loads, where only little shortening occurs, have a higher oxygen consumption than isometric twitches, while isotonic twitches with small loads and consequently large shortenings need less oxygen than the isometric twitches.

4. The value of 0.380 for the oxygen consumption per gram tension and unit of muscle length is in good agreement with the corresponding values of heat measurements. The maximal efficiency observed was 13.8 per cent.

5. From the experiments the conclusion is drawn that the energy liber-

ated in a twitch depends on two factors, the length of the fibres during contraction and the work done (Fenn-effect).

6. It is suggested that the length of the fibers influences much more the energy change for developing the tension than the energy needed for maintaining the tension. This difference explains why it is much easier to find the Fenn-effect in tetanic contractions.

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THE HEMOGLOBIN CONTENT OF THE BLOOD OF FOWLS

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The primary purpose of this study was to increase our knowledge of the hemoglobin content of the blood of the chicken. We are also able to report a study of the hemoglobin content of the blood of a limited number of wild gallinaceous and aquatic fowls.

LITERATURE. The literature contains only meager data on the hemoglobin content of the blood of the chicken. Calculations made by Preyer (1871) on the basis of iron determinations of previous workers indicate, when averaged, that the blood of the chicken has a hemoglobin content of 10.1 grams per 100 cc. Müller (1886) by a spectroscopic method found that the blood of the chicken contains 16.75 grams of hemoglobin per 100 cc. A chick a few days old had a content of only 6.91 grams per 100 cc. Fritsch (1920), using the spectrophotometer in a study of 10 chickens, found the average hemoglobin content of the blood of cocks to be 12.3 grams per 100 cc.; of hens, 9.6 grams per 100 cc. Hart, Elvehjem, Kemmerer and Halpin (1930), using the Newcomer method, concluded that the normal hemoglobin content of the blood of chicks is between 6 and 7 grams per 100 cc.

We have not found any record of hemoglobin determinations in wild fowls.

MATERIALS AND METHODS. The hens, pullets and cocks used in this study belonged to the College Poultry Husbandry Department and were kept under the conditions prevailing at their poultry farm. The wild fowls belonged to the State Fish and Game Department and were kept under the conditions current at their game farm.

The mean age of the hens was 658 $\pm$ 10 days. The pullets ranged in age from about 4 to 6 months. The cocks and all wild fowls, except a few turkeys, were mature.

A blood sample was obtained from a wing vein of each bird. The hemoglobin determinations were made with a Bausch and Lomb "Improved Newcomer Model" hemoglobinometer incorporating the Newcomer (1923) acid hematin method. The readings of the instrument are in grams per 100 cc. of blood. The blue filter provided with the hemoglobinometer was used in making all readings. The source of light was a colorimeter lamp

equipped with a blue-glass 60-watt Westinghouse Mazda bulb. Although the manufacturers state that in calibrating the hemoglobinometer readings were made against a north sky, we found that there were no significant differences between readings when daylight was used and readings when light from the colorimeter lamp was used. The hemoglobinometer was standardized by making hemoglobin determinations on 18 apparently healthy men ranging in age from 20 to 27 years, and comparing the results with the well known and widely accepted spectrophotometric findings of Williamson (1916). The average hemoglobin amount in our group of men was 16.98 ± 0.14 gram per 100 cc. Williamson's average for a group of men ranging in age from 21 to 25 years is 17.23 grams per 100 cc. We therefore consider our instrument to be accurate (except for turbid acid hematin solutions).

A correction for turbidity. Acid hematin solutions prepared from mammalian blood in accordance with the Newcomer method show a turbidity which causes the hemoglobin readings to be too high (Terrill, 1922). Similar solutions prepared from bird blood show a much higher turbidity, doubtless chiefly caused by the presence of the nuclei of the more or less completely hemolyzed erythrocytes.

Having found in the literature no turbidity corrections for bird blood solutions prepared for hemoglobin determination by the Newcomer method, we made such corrections in the following way, using the "Improved Newcomer Model" hemoglobinometer:

A hemoglobin reading on a preparation of bird blood was made in the usual way, and then a preparation of mammalian blood of high hemoglobin content (human blood) was diluted with water until it gave the same hemoglobin reading as the bird preparation. These acid hematin solutions were then transferred to a Klett nephelometer where the one from the mammal was made as turbid as the one from the bird by adding a suspension of killed bacteria (Brucella abortus). The mammalian sample, now turbid, was returned to the hemoglobinometer where another reading was made. From this reading the hemoglobin value of the bird (or non-turbid mammalian) sample was subtracted and the difference was considered to represent the correction for the turbidity of the bird sample over and above the turbidity of the mammalian sample. To test the limit of error of the method, duplicate determinations were made on one fowl and triplicate determinations on another, and it was found that the respective results agreed closely.

By this method we determined the turbidity correction on the blood of 23 chickens including 20 hens, 2 cocks and a two-month-old chick. The average results for the group are as follows: Uncorrected hemoglobin reading of bird samples and hemoglobin reading of diluted non-turbid mammalian samples, 12.97 grams per 100 cc.; hemoglobin reading of mammalian

samples after being made turbid, 15.63 grams per 100 cc.; difference, i.e., turbidity correction for bird samples, 2.66 grams per 100 cc.; corrected hemoglobin reading of bird samples, 10.31 grams per 100 cc.

The correlation between the corrected and uncorrected hemoglobin readings was found to be 0.97 ± 0.01. The equation for converting uncorrected readings into corrected readings is

$$C = 0.91U - 1.49,$$

where C is the corrected reading and U the uncorrected reading. A scale for converting uncorrected readings into corrected readings is shown in figure 1.

Evidently the turbidity for which we have corrected is the turbidity of bird acid hematin samples in excess of the turbidity of the mammalian samples used in the nephelometer against the bird preparations. Some preliminary observations indicate that the correction represents two-thirds or more of the turbidity of the bird acid hematin samples.

Having made some studies of the hemoglobin content of the blood of wild fowls, we were anxious to know if the same correlation that exists for the uncorrected and corrected hemoglobin values of the chicken holds also for birds of other species. We submit the following evidence in support of the view that the turbidity-correction data derived from the chicken have wide applicability to birds:

We measured the hemoglobin value and the turbidity (by the nephelometer) of acid hematin solutions prepared from each of 14 chickens and found the correlation between hemoglobin content and turbidity to be 0.67 ± 0.09; the correlation between corrected hemoglobin and turbidity to be 0.58 ± 0.09; and the correlation between correction and turbidity to be 0.69 ± 0.09. We also measured the hemoglobin value and the turbidity of acid hematin solutions prepared from each of 12 wild fowls (pheasants, Canadian geese and mallards), using as the standard in the turbidity measurements an acid hematin sample from a typical hen as regards turbidity and hemoglobin, and found the following correlations: Uncorrected hemoglobin and turbidity 0.90 ± 0.04; corrected hemoglobin (according to the correction formula for chickens) and turbidity 0.90 ± 0.04; turbidity correction and turbidity 0.89 ± 0.04.

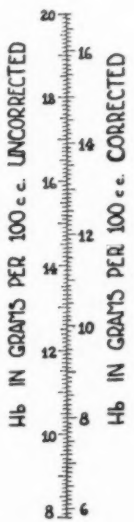

Fig. 1. Scale for converting uncorrected hemoglobin readings into corrected readings.

Additional evidence that the turbidity-correction data of chickens are applicable to the wild fowls mentioned above is seen in the fact that these birds show red corpuscle counts that are in general no higher than counts made on chickens.

RESULTS. *Chickens.* The mean hemoglobin content of the blood of the chickens studied is shown in table 1.

The distributions based on hemoglobin content are remarkably close to the normal.

Wild fowls. The average corrected blood hemoglobin values for the wild fowls studied are as follows (grams per 100 cc.): pheasants, 13.7; ducks, 14; geese, 14.9; brants, 14.7; swan, 13.4; peafowls, 12.0; turkeys, 10.7.

TABLE 1

Mean hemoglobin content of the blood of chickens

BREED AND SEX	NUMBER OF INDIVIDUALS	UNCORRECTED HEMOGLOBIN	CORRECTED HEMOGLOBIN
		gm. per 100 cc.	*gm. per 100 cc.*
White Leghorn hens	101	12.8 ±1.0	10.2 ±0.9
White Plymouth Rock hens	101	12.3 ±0.8	9.8 ±0.7
Rhode Island Red hens	102	11.9 ±0.7	9.4 ±0.7
All hens	304	12.3 ±0.1	9.8 ±0.1−
White Leghorn pullets	101	11.4 ±0.7	8.9 ±0.7
Cocks (mostly White Leghorns)	26	16.4 ±0.2	13.5 ±0.2

Sex differences were slight and inconstant; however, the number of birds studied was small.

Werner (1928), using the spectrophotometer, found that the domestic duck and goose have the following amounts of hemoglobin in the blood (grams per 100 cc.): drake, 13.8; duck, 12.2; gander, 12.1; goose, 13.0.

SUMMARY

A study of the hemoglobin content of the blood of the chicken and of wild fowls by the Newcomer method is reported.

A correction applying to the "Improved Newcomer Model" hemoglobinometer is offered for the turbidity of acid hematin solutions prepared from bird blood.

The hemoglobin content of the blood of wild fowls is higher than the hemoglobin content of the blood of chickens.

The statistical work was done by Prof. A. E. Brandt of the Mathematics Department to whom grateful acknowledgment is made.

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THE METABOLISM IN PREGNANCY

V. The Carbohydrate Metabolism[1]

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In previous papers certain material has been presented dealing with the nitrogen metabolism (1) of the normal pregnant woman, based upon an investigation progressively conducted since 1921. Briefly reviewed, the subjects for the study have been drawn from (*a*) the patients presenting at the Prenatal Clinic of the Robinson Memorial, supplemented by a few volunteers referred from private sources, and (*b*) the inmates of two nursing homes for unmarried mothers. A wide scatter of dietary and hygienic habit was thus secured. The studies of each individual were carried on continuously both ante- and post-partum as by so doing both the directions and amounts of recorded changes assume a real significance. Pertinent details concerning the composition and physical characters of the several groups are given in the paper already cited and need not be repeated here.

The present paper deals with certain phases of the carbohydrate metabolism, namely, urine and blood sugars, and the galactose tolerance.

Urine sugar. The occurrence of sugar in the urine of pregnant women has been recorded by many and the fact subjected to a wide variety of interpretations ranging from the existence of diabetes to a physiologic relief. Lack of reliable chemical methods for the detection of urine sugar vitiates many of the statistics of the older literature, and more recent papers range from an incidence of less than 1 per cent (2) to 18 per cent in a report of the senior author (3). In a very recent report (4) to the original of which we have not had access, the author reports lactose in 40 per cent of the cases after the mammary glands have begun activity, and adds that glucose is very common. As he finds acetone in 10 per cent of his group, the question of the normality of a part of the pregnancies at once comes into consideration.

The paper from the senior author already cited (3) includes a report of the occurrence of melituria ante- and post-partum, and this pertinent

[1] Presented, in part, at the Annual Meeting of the American Physiological Society, Chicago, Ill., April, 1930.

material can be presented briefly in tabular form. The presence of reducing sugar was determined by the well-known Benedict reagent and when found, the amount determined by the quantitative procedure of the same investigator. Glucose was verified by fermentation, and irregularly by osazone formation, lactose by a negative response to Barfoed's reagent, a positive mucic acid test, and in a number of the specimens the osazone was formed and identified. The data from this series are given in table 1.

But few comments are necessary. The reports from group I involved the examination of over seven hundred twenty-four-hour collections. The use of specimens on the day before delivery may have depressed the number of positive responses although the figure agrees well with that reported by Peco. An intermittency in lactose excretion at this time seems improbable even though our data do not specifically exclude it. Needless to say, in all cases where specimens and not twenty-four-hour collections

TABLE 1

Ante- and post-partum melituria

GROUP	DESCRIPTION	MATERIAL	NUMBER OF CASES	PER CENT POSITIVE	SUGAR
				per cent	
I	During pregnancy, 4th–9th month	24-hour collection	100	18	Glucose
II	1 day before delivery	Specimen	50	38	Lactose
	3 days after delivery	Catheterized specimen	50	100	Lactose
	4 days after delivery	Catheterized specimen	50	100	Lactose
	10 days after delivery	Catheterized specimen	50	92	Lactose
III	14 days after delivery	24-hour collection	50	82	Lactose
IV	6 months after delivery	24-hour collection	50	80	Lactose

were examined every care was exercised to avoid the disturbing effect of glycuresis. The lactosuria, susceptible to explanation as the result of over-production, does not immediately concern the present thesis. The glycosuria of pregnancy, on the other hand, is wholly germane to the problem in hand. The demonstration of a lowered sugar assimilation limit as a sign of pregnancy has been suggested by several, but all such procedures have been shown to be both positively and negatively misleading. Confidence in a single test which is susceptible to a wide variety of unrelated controlling influences (5) errs on the side of an uncritical optimism, when applied to the definition of one of them.

In the series of thoroughly studied cases which forms the basis of this present report, twenty-seven or 35 per cent, showed sugar at some time during the ante-partum period. To avoid a possibly misleading factor, all examinations during the last week before delivery have been excluded

in arriving at this figure. This value is materially superior to other records, even the high value of our own previous report. Better control of material and more frequent and consistent examinations are undoubtedly factors in this result. Only fourteen, or 18 per cent, showed repeated recurrence of the glycosuria, and with the majority of these, there were aglycosuric intervals throughout the course of the pregnancy. Typical protocols are given in table 1A. Patently, where but few specimens from the individual case—frequently one only—are examined, the probability of collection during an aglycosuric interval is increased; we feel that this consideration offers an explanation of the discrepancy with other reports.

To summarize, the intermittent appearance of glucose in the urine during a normal pregnancy is an event of fairly common occurrence and *in itself* is to be regarded as no more than one expresssion of the underlying physiological condition. This generalization naturally does not apply to those cases in which other observations demonstrate the presence of some pathological condition coexistent with the pregnancy. Among these would be certain of the endocrinopathies including diabetes, and in addition, syph-

TABLE 1A

Protocols of glycosuria patients

		WEEKS A.P.														
		27	22	18	17	15	3	12	11	10	9	8	7	5	4	3	2
Glucose (gms.).	A-21	0	s.t.*	0		0	5.2		0	3.0	5.4		2.6	0.3		0	
	C-23				3.6	s.t.		1.3		2.8		s.t.		s.t.	3.6		s.t.

* Slight trace.

ilis, lesions of the central nervous system, hepatic dysfunction, primary anemia, and the leukemias, to name but a part. Evidences other than the glycosuria would be the factors determining such diagnoses.

Blood sugar. With glycosuria a not unusual observation in the course of an apparently normal pregnancy, attention naturally turns to the sugar levels of the blood for possible correlating phenomena. While the threshold for sugar suggested by Jacobsen (6) has been repeatedly demonstrated to be at best a highly mobile barrier, the concept persists in a variety of modified forms and many writers still affirm a causal interrelation between the level of blood sugar and the appearance of glucose in the urine.

Blood sugar determinations were a part of the routine study. The original Folin-Wu method (7) has been used throughout in order that the measurements made over a period of ten years should remain strictly comparable. The data from this portion of the study are collected in table 2.

Obviously, the glycosuria cannot be traced to hyperglycemia, the average levels throughout the ante-partum period showing a uniformly low normal

value. While the level in the last week before delivery is the lowest recorded, it fails to define any special downward tendency. A number of individual measurements in the series fell below the conventional lower limit of 80 mgm. but not to any marked degree, as 70 mgm.—and that but once—was the lowest level recorded. Equally, the highest value was 100 mgm. In the interest of conserving space no attempt is made here to review the fairly extensive and highly contradictory literature on this subject. The factual evidence from this study is presented as typical of this group of pregnant women, the general physical normality of whom was a matter of objective investigation and not of assumption.

A further evidence of the absence of hyperglycemia in determining glycosuria is shown in the individual case protocols. Selecting case C-23

TABLE 2

Blood sugar averages

ANTE-PARTUM		POST-PARTUM	
Weeks	Blood sugar	Weeks	Blood sugar
	mgm.		*mgm.*
Over 24	84	1	
20–21	86	2	
20–17	83	3	93
16–13	83	4	94
12–9	84	5–8	93
8–5	83	9–12	95
4	82	13–16	95
3	86	17–20	92
2	82	21–24	99
1	81	Over 24	98
Average (weighted).....	83		94

from table 1 as typical, the several blood sugar readings corresponding to the urine reports were severally 79, 80, 84, 80, 76, 86, 81 and 79 mgm. The highest and lowest values each correspond to a glycosuria reported as a "slight trace."

With the termination of the pregnancy, there is an obvious rise in the blood sugar levels to those that can be regarded as more nearly midway in the normal zone. Unfortunately, our data fail to define the conditions during the immediate transition period. Our primary concern lies with the ante-partum interval, and because of the patient's condition, studies during the earlier days after confinement must be very incomplete. For this reason, all measurements were omitted even with those patients to whom we had access (groups A and B) at this time.

To summarize briefly, blood sugar levels during the course of normal pregnancy tend to assume low normal or slightly sub-normal values.

Beginning two weeks post-partum, at least, there is a consistent rise which assumes and determines wholly normal levels. There is every evidence that the ante-partum glycosurias are in no way produced by hyperglycemia.

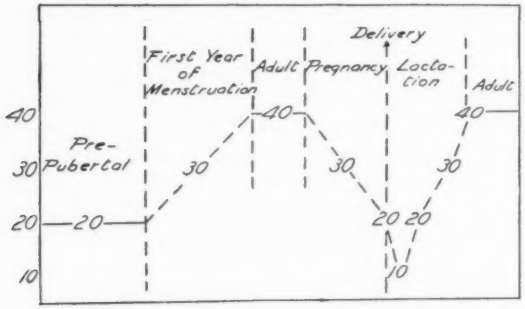

Fig. 1. Influence of sexual status on tolerance level.

TABLE 3

Ante- and post-partum changes in galactose tolerance

PERIOD	MONTH	TOLERANCE DOSE		
		20 grams	30 grams	40 grams
		per cent	*per cent*	*per cent*
Ante-partum	3	67	33	0
	4	67	33	0
	5	63	37	0
	6	67	33	0
	7	62	38	0
	8	89	11	0
	9	100	0	0
Delivery				
Post-partum	1*	100	0	0
	2	73	27	0
	3	100	0	0
	4	67	33	0
	5	25	75	0
	6	0	50	50
	Over 6	0	0	100

* 3rd and 4th weeks only.

Galactose tolerance. For a number of years, one of us in conjunction with various associates, has been conducting investigations on the metabolism of galactose. As the greater part of this material has already appeared in the literature (8, 9, 10, 11, 3, 12, 5), no extensive review is

here necessary. That portion of the results bearing on the influence of pregnancy can be presented most compactly in the accompanying diagram.

Briefly expounded, it may be said that the tolerance dose of the healthy adult female is 40 grams. With the inception of pregnancy, there is a decline in tolerance, the rapidity of progress depending on the individual case, but all ultimately reaching the constant level of 20 grams, which is that of the prepuberal years. After delivery, for a short time (2 weeks or more) the level sinks still further to 10 grams—presumably an expression of the saturation of the mammary storage capacities with galactose. Following this,there is an upward trend, and with the cessation of lactation, or even before, the normal adult level of 40 grams is attained.

The data from the present series of patients are collected in table 3.

The dominant tendency even during the early months of pregnancy is toward the final level but is manifested uniformly only in the last few weeks. Recovery after delivery is irregular, the upward trend exhibiting itself early in the post-partum phase but becoming established at the original level only after the lapse of months. Lactation, with its highly specialized functions which include the active synthesis of galactose, undoubtedly plays the dominant rôle in the slow recovery.

In eight cases studied within a few days (5 to 7) after spontaneous or induced miscarriage in the second to fourth month, three were positive with 20, four with 30, and one with 40 grams.

SUMMARY

This paper may be briefly summarized as follows:

1. Glycosuria is a common finding during the course of normal pregnancy. Lactosuria supervenes within a few days of delivery and may continue throughout the period of lactation and even beyond.

2. Blood sugars are at low normal or slightly sub-normal levels throughout pregnancy. They return to mid-normal levels early in the post-partum period.

3. The galactose assimilation limit is depressed by normal pregnancy to the level of the prepuberal tolerance. After delivery there is a slow recovery to the normal adult level, the retardation being influenced, in part at least, by the synthesis of galactose by the mammary glands during the lactation period. The normal tolerance may appear, however, before the cessation of lactation.

Thanks are due and gladly rendered to the members of the institution staffs and to the patients who coöperated in this study.

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THE METABOLISM IN PREGNANCY

VI. The Respiratory Metabolism and Acid Elimination[1]

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From the Evans Memorial, Boston, Mass.

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In a series of previous communications, the authors have presented certain results of an investigation on various phases of metabolism during normal pregnancy. The composition of the several series, and the nitrogen (1) and carbohydrate (2) metabolism, have already been considered and need not be touched upon here.

The present paper deals with the respiratory metabolism, with the concomitant control physical measurements, the lung volume, the tension of alveolar carbon dioxide, and some aspects of the acid-base elimination. These several topics can be considered seriatim.

Basal metabolic rate. In a preliminary paper (3) the senior author reported the results of consecutive studies on forty-six pregnant women from an average of twenty-one weeks before to five weeks after delivery. To these data have been added the records of thirty-one additional cases, the average period of study of which has been fourteen weeks both ante- and post-partum.

Prior to the appearance of this earlier paper, the literature contained four complete studies on single cases (4), (5), (6), (7), an incomplete record from three cases (8), and an intensive study on three women during three weeks before delivery, and both the mothers and children for two weeks after this event (9). In addition, two other papers recorded isolated measurements on series of pregnant women (10), (11), but these were vitiated by a lack of basality, a fact recognized by the second author. Since that date, additional reports have appeared (12), (13), (14), (15), (16).

Summarized, all of the records agree that there is an increase in the respiratory metabolism during the progress of the pregnancy and to an amount definitely in excess of that conditioned by the augmentation in weight. Less in accord are the actual increments reported from the

[1] Presented, in part, at the Annual Meeting of the American Physiological Society, Chicago, Ill., April, 1930.

several sources. The allocation of the origin of this increase solely to the fetal metabolism, as suggested by Sandiford and Wheeler (7), rests primarily on a most uncertain extrapolation applied to the data of a single case. The question—frankly a moot point—need not be debated here as the immediate thesis is an objective record of the changes in the basal rate produced by pregnancy from those of an equivalent female in a state of complete sexual rest. The curve of change during normal pregnancy can then serve as a base line for comparison with the rates recorded in the several toxemias which may be incident to the initial condition.

A few details of method and procedure may be briefly described. As has been shown in an earlier paper (1), none of the patients gave evidence of an inadequate protein intake.

The subjects in group I, private cases and patients from the Prenatal Service of the Robinson Memorial, reported at the laboratory in the early morning in a post-absorptive (fasting) state. The patient, after emptying the bladder, was placed in a recumbent position with loosened clothing and adequately covered for a period of not less than thirty minutes and as much longer as was necessary to secure relaxation as shown by repeated concordant measurements of the pulse rate. Two or three—never more—ten-minute independent measurements of the oxygen consumption were made with brief rest intervals between each. Frequent pulse, blood pressure, temperature and respiration measurements were made throughout the period of study, and at the end the nude weight was measured on an accurate scale and the standing height recorded with standard calipers.

The patients in group II were inmates of a nursing home and were moved from their own to the metabolism room and then subjected to exactly the same procedure as that outlined above for group I.

The remaining group (III), also inmates of a nursing home, were allowed to remain in the bed in which they had slept, and the metabolism machine was brought to them. Frequently they were awakened by the entrance of the operator. The subsequent procedure was identical with that for the other groups.

The older type of Benedict-Collins (17) respiration apparatus with internal motor has been used throughout. The objections offered to it reflect primarily on the care which the instrument has been given. The expenditure of not over five minutes a day in breaking down, cleaning, and reassembling the apparatus will eliminate with certainty the features to which exception has been taken. In conserving the comfort of the patient and lessening the labor of respiration, it is certainly superior to the valve type of machine. This opinion rests on the study of over fifteen thousand cases and a careful control study with a standard valve machine. The

usual checks on the mechanical accuracy of the instruments were applied at frequent intervals. Soda lime was changed when half of its calculated absorbing capacity was exhausted and the validity of the last previous measurement checked by a barium hydroxide test. At intervals during the study, the absorbing power of the soda lime was measured independently. The highly debatable question of choice in the use of open or closed circuit methods is not germane to this thesis. The uniform use of a single method is essential if results are to be rigorously comparable; the authors' selection of the closed circuit method was made on the basis of extensive experience.

The observed rates were calculated from the oxygen consumption, following the usual convention of assuming an R.Q. of 0.82. The existing records in the literature indicate that this is as warrantable in normal pregnancy as in the other conditions in which it is routinely adopted. Comparisons were made with the predictions both by the Harris-Benedict (18) formula and that of Aub-duBois, (19) and the mean value accepted as that of the measured deviation. As might be expected from the character of the subjects, the correlations between the two comparisons were excellent.

The data can be presented most compactly in graphic form. The averages of the three series were computed separately and the individual curves defined by each recorded on the graph.

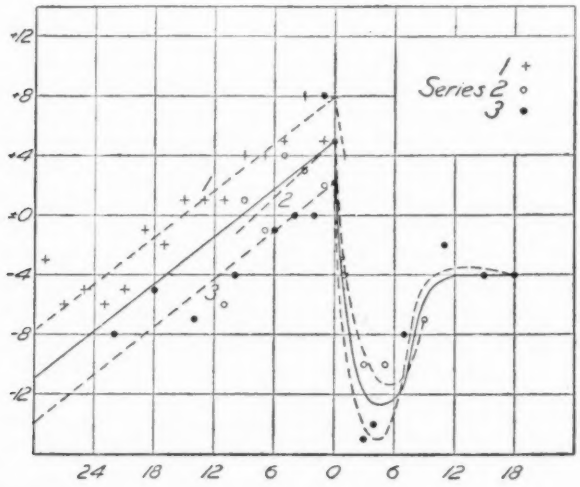

Fig. 1. Change in basal rate deviations throughout ante- and post-partum periods

In the ante-partum period, series I shows a curve some 3 per cent higher than that of series II, while the third series is 3 per cent lower. The slope of the three curves is substantially the same, that of the two series with the longer observation periods being practically identical. Such correlations between independent series in which subjects, observers, machines, and chronology were all completely different, warrants some confidence in the representative character of the curve. The uniformly lower values attained with series III would seem to imply a truer basality in this group as the conditions preliminary to a measurement were somewhat more conducive to a complete relaxation. Some support for this explanation lies in the position of the curve for series II, these patients falling short of the ideal conditions which obtained with III but manifestly superior to those governing series I. The difference in any case is not of a disturbing magnitude, and the two extreme curves may be felt to represent the extremes of current practise. For this reason, it is regarded as warrantable to summarize all of the data, and plot a curve of average performance—shown by the solid line in the graph—to serve as the base line for future comparisons. To simplify future use, these data may be reduced to tabular form, and for convenience sake, those from the post-partum characteristic are included (table 1).

Extrapolations beyond twenty-four weeks ante-partum are felt to be uncertain and are omitted from the table. The average value for a control group of pregnant women, studied in connection with an independent investigation, was −6 per cent.

Turning to the post-partum interval—included in the study for the sake of completeness and as a control—it is evident that there is a profound drop in the deviation which reaches a maximum at some time from the third to the fifth week. Again the correlations of the data from series II and III are excellent, and the differences are in the same sense as those in the ante-partum period. By the end of the third month post-partum, the average rate is 4 per cent below prediction, and, so far as the observations continue, remains at this level. With the lack of absolute definition of the normal rate which necessitates the establishment of a zone of normality properly to absorb variations intrinsic in normal individuals, the curves as plotted establish a base line of reference both ante- and post-partum which should allow of a more just interpretation of the observed data of women in these conditions.

Physical measurements associated with basal rate determinations. At the risk of criticism for an ex-cathedra generalization, the authors wish to affirm that no basal rate report can be regarded as complete which fails to record pulse and respiration rates, body temperature, and blood pressure. Many times these concomitant physical measurements give objective evidence of a lack of basality which would completely fail of recognition

were the deviation of the rate alone recorded. Further, single measurements do not suffice to establish the true condition but should be repeated at intervals throughout the test. A routine which has been found to yield satisfactory results is as follows:

Temperature
Blood pressure
Basal rate { Pulse records, 3
Respiration records, 3
Blood pressure
Basal rate { Pulse records, 3
Respiration records, 3
Blood pressure
Temperature

When the kymograph is not used—and there are certain valid objections to its substitution for direct observations, at least in clinical practise—the indicator travel can be estimated and an approximate value for the minute volume computed.

TABLE 1

Deviations from normal prediction produced by pregnancy

	WEEKS								
	24	21	18	15	12	9	6	3	0
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Ante-partum	−8	−6	−5	−3	−1	±0	+2	+4	+5
Post-partum	−	−	−4	−4	−4	−5	−12	−12	−

In the present study, blood pressures were measured by a mercury column connected with a broad soft cuff and using an auscultatory tambour, the pulse and respiration rates counted with the stop watch, and temperatures taken orally with calibrated mercury thermometers. That this last procedure leaves something to be desired is recognized, but its use was conditioned, as were the other compromises in the investigation, by the necessity of maintaining the coöperation of the subjects.

As no real trends of change were observed with certainty, only averages for the two periods are reported. The similar averages from the control group already mentioned above are included (table 2).

A pulse rate slightly above the average, a temperature ante-partum slightly superior to the post-partum level, and a downward tendency to the blood pressure, which has long been recognized as characteristic of pregnancy, are the only points of notice. The correlations with the control group are satisfactory.

Lung volume. Some years ago, Dreyer (20) published a series of prediction tables permitting, among other things, the calculation of that lung

volume which could be regarded severally as normal for a given sitting height, chest girth, and body weight. As his data were drawn from individuals approximating a normal body configuration, departures from this latter in the emaciated, and even more significantly in the obese, lead to extrapolations in the two latter correlations that yield impossible magnitudes. That for sitting height, however, has proven useful in dealing with even extreme cases. West (21) has also offered prediction formulae based on standing height and on body area which have also yielded serviceable criteria, though the second is also unduly influenced by extremes of nutritional status.

For the measurement of the sitting height it has been found necessary to modify Dreyer's original definition of the posture to be assumed as the patients found it difficult and even impossible to sit on the floor. We have fixed the measuring calipers firmly to a stool, at first 13 inches, later 10 inches high and 12 inches square. The buttocks are pressed firmly against the back board, the lower leg is vertical, and thus the knees are elevated

TABLE 2

Physical measurements

	PARTUM		CONTROLS (ANTE-PARTUM)
	Ante-	Post-	
Pulse	78	67	77
Respiration	17	17	17
Temperature	98.1°	97.8°	98.3°
Blood pressure:			
Systolic	108	112	107
Diastolic	67	70	63

slightly. This seems to reduce the gluteal variation to a minimum and yields satisfactorily reproducible results.

The lung volume was measured with a spirometer of standard type with counterbalanced bell. Flattened glass mouth pieces of large bore aided in avoiding leakage. The lung volume is unfortunately a measurement peculiarly at the mercy of the degree of the patients' coöperation. Myers (22) has stressed this point in his excellent monograph on the subject.

For this reason, consecutive records lack the uniformity obtainable in observations not under the voluntary control of the patient. The average results from the combined series are given in table 3.

A steady upward trend is unmistakable, though the increments during the last months show a lessened magnitude. The striking feature of the increase lies in the fact that the anatomical conditions, a priori, would seem to conduce to a downward trend. It is to be noted that all of the averages fall short of Dreyer's normal prediction based upon sitting height, even

though the moderate values of his standard "B" are selected. We feel that the element of coöperation plays a large part here. The trend is certain and significant; the absolute values important only as they define criteria for this condition. The average value from the control group was −16 per cent.

Alveolar carbon dioxide. In an earlier paper, (23) the senior author gave certain preliminary results from observations of this magnitude, confirming the low values in pregnancy earlier recorded by Hasselbalch (6) and others. The interesting feature is the lowering of the tensions of alveolar carbon dioxide to levels usually associated with acidosis in patients who give no other evidences of an acid intoxication. During the earlier study (l. c.) both the Marriott (24) and Fredericia (25) methods were compared and both standardized against analyses with the well-known Haldane apparatus. They were found to be equally reliable when the original

TABLE 3
Lung capacity

PERIOD	LUNG VOLUME	DEVIATION FROM NORMAL*
weeks	*cc.*	*per cent*
Over 24	2200	−27
24–21	2390	−21
20–17	2460	−19
16–13	2530	−17
12–9	2630	−13
8–5	2670	−12
4–3	2680	−11
2–1	2700	−11

* Based upon Dreyer's Sitting Height prediction, "B" standard.

Marriott apparatus was modified to give a more adequate passage for the air exchange. The Fredericia, giving arterial tensions, was finally selected in preference to the Marriott, which measures the venous levels, on the grounds of simplicity and greater ease of sterilization. The averages from the combined series lend themselves to graphic representation, and are presented in this form.

The record shows a uniform depression of the tension to a level definitely below the conventional inferior normal limit of 35 mm. The average for the control group was 31 mm. Further, with the expulsion of the fetus, there is a progressive upward tendency that at the end of four weeks brings the average within normal limits. The cause of this phenomenon is obscure; several hypotheses have been offered, but none have received general acceptance. With the recognized disturbance in carbohydrate metabolism which accompanies pregnancy, the first thought is of a ketosis, but these normal patients do not show ketone acids in the urine in

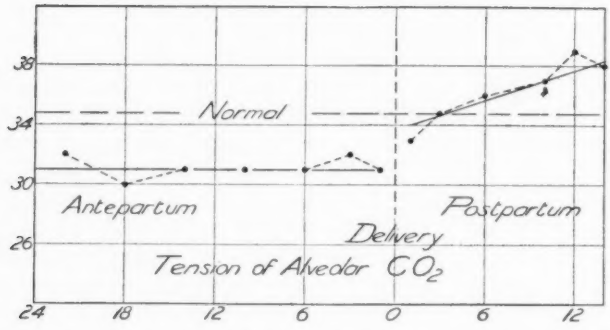

Fig. 2. Tensions of alveolar carbon dioxide (arterial) in millimeters of mercury both ante- and post-partum.

significant amounts and are free from the clinical signs which accompany an acid intoxication. The combining power of the plasma is lowered (see Stander (26) for bibliography) and this in turn should possibly imply a loss of fixed alkali through the kidneys. In an earlier paper in this series (1) attention was called to the fact that no marked increase in the ammonia output is observed (5.0 per cent), and while the value is absolutely somewhat high, it is maintained at this level (5.1 per cent) during the postpartum period in which the carbon dioxide returns to normal even while the carbohydrate tolerance is still at a subnormal level. The pH values of the blood undergo but insignificant change and that only in the terminal phase of the pregnancy.

If an acidosis exists, it is obviously compensated. In the hope of throwing some light on the matter, certain of the mineral constituents of the urine were determined as a part of the routine.

The total acid of the urine can be readily computed, as suggested by Fitz and van Slyke (27), from the sum of the titratable acid (Folin, 1905) (28), and the ammonia content expressed in terms of the N/10 acid which it may be assumed to have neutralized. Phosphates were titrated by the standard uranium nitrate procedure, and chlorides with silver nitrate and thiocyanate. The latter is expressed as sodium chloride, a not wholly accurate convention, but it gives a rough measure of an important moiety of the fixed alkali in the urine. The data from these analyses are given in table 4.

Standards for the normal level of acid excretion are not well defined, but equivalent values ranging from 500 to 900 cc. of N/10 acid are usually accepted as within normal limits. The values given here tend toward the lower level, but the averages ante- and post-partum are both within the normal range and are practically identical.

Normal phosphate elimination is even less well defined, although an elimination of from 2 to 2.5 grams calculated as P_2O_5 is generally accepted; experience has shown maintenance on ingestion of these amounts. During the ante-partum period the values recorded fall below the inferior limit of 2.0 grams but storage is going on, in significant amount at least, during the later stages. It is a possible assumption that the urine phosphorus is no fairer criterion of this phase of metabolism than is the urine nitrogen of the protein intake. Post-partum there is an increase in the phosphate elimination that brings the average value just above the arbitrary low boundary of the normal.

TABLE 4

Acid and base elimination

PERIOD	ALVEOLAR CO_2	TOTAL ACID	P_2O_5	NaCl
weeks	*mm. Hg*	*cc. N/10*	*grams*	*grams*
Over 24		510	1.56	13.8
24–21	32	570	1.82	10.3
20–17	30	590	1.80	10.3
16–13	31	510	1.60	10.4
12–9	31	600	1.76	10.8
8–5	31	630	1.70	10.1
4–3	32	650	1.90	10.9
2–1	31	580	1.77	8.7
		Delivery		
1–2	33	(890)	2.16	9.8
3–4	35	650	2.26	7.3
5–8	36	520	2.09	9.8
9–12	37	530	1.65	10.4
13–16	39	560	1.99	10.4
Over 16	38	570		9.4
Average A.P.....	31	580	1.74	10.7
Average P.P.....	36	570	2.03	9.5

The question remains unresolved. A low-grade phosphoric acid retention could be one interpretation; equally, the amounts recorded could be regarded as normal if the known tissue storage were conceded to be a factor.

The sodium chloride values show an opposite trend. The values during both periods are within the probable normal range; the higher values ante-partum may be associated solely with the larger fluid output during this period. There is certainly no reliable evidence of a significant increase in the elimination of fixed alkali.

Considering all the available data, while they might be interpreted as indicating a slight acid retention during pregnancy, the values obtained do

not present convincing magnitudes, and all the trends exhibited are susceptible of equally plausible explanations that exclude an acidosis. Careful complete metabolism studies will be necessary to resolve this question, and one despairs of securing that degree of continuous coöperation from the nervously unstable gravid woman.

SUMMARY

The facts in this report may be briefly summarized as follows:

1. Pregnancy produces an increase in the basal rate, during the last twenty-four weeks, of 13 per cent more than that predicted from the change in weight. Following delivery there is a downward trend of significant proportions which reaches a minimum value somewhere between the third and fifth week and then rapidly rises to a level within the normal zone. With the exception of the post-partum depression, the average deviation values fall within conventional normal limits.

2. The pulse rate is slightly increased, the temperature relatively so, and the blood pressure moderately depressed in normal pregnancy.

3. The lung volume shows an absolute upward trend throughout the ante-partum period, although the growing uterus would seem likely to produce an opposite effect. The values recorded are all below normal prediction levels but are not regarded as wholly dependable as they are subversively influenced by the measure of the patients' coöperation.

4. Alveolar carbon dioxide tensions assume values normally associated with acid intoxication. Such a condition cannot be demonstrated with certainty, however, although there are minor evidences of possible acid retention. The present data offer no real help to a solution of the problem.

The authors extend their thanks to the hospital staffs and to the patients who have coöperated in this study.

It is a pleasure also to acknowledge indebtedness to the American Association for the Advancement of Science for generous aid which greatly facilitated one portion of the study.

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THE METABOLISM IN PREGNANCY

VII. The Blood Morphology[1]

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In a recent communication (1) the author and his associates have reported the results of studies in normal pregnancy dealing with the nitrogen metabolism. Reference is made to this paper for full details as to the composition of the group, the methods of study, and other significant details.

The present paper deals with the changes in the blood morphology recorded in a series of seventy-seven patients studied consecutively both ante- and post-partum. The longest single series of observations was thirty-four weeks ante- and thirty weeks post-partum; the averages for the entire group, fifteen and eight weeks respectively. Hemoglobin was determined by the Dare method; the blood counts were made with the usual standard apparatus which had been calibrated.

Chronological analyses failed to disclose any certainly significant trends This is probably due to the relatively small number of cases which did not suffice to absorb individual differences. In Galloway's (2) excellent paper on anemia, based upon a much larger series, there was a progressive fall in both hemoglobin and erythrocyte count. His figures may be abstracted (table 1).

Since our own figures fail to establish progressions, only averages for the periods ante- and post-partum need be presented. Following the practice of the earlier papers, the average results from a group of pregnant women studied in connection with another investigation are included as a control. The data are collected in table 2.

The secondary anemia ante-partum, so frequently recorded by others, is clearly shown by these averages. The lowering in the erythrocyte count could be explained by an increase in blood volume of the order of 10 per cent—a figure in accord with other records. The hemoglobin, however, shows a depression that definitely exceeds any dilution effect and which is disproportionately greater than that shown by the erythrocytes.

[1] Presented, in part, at the Annual Meeting of the American Physiological Society, Chicago, Ill., April, 1930.

The control group shows the same tendency though in less marked degree. It seems reasonable to conclude that there is an absolute lowered hemoglobin content during pregnancy, and at least a relative diminution in the erythrocyte count. After delivery there is a definite upward tendency to both factors, the hemoglobin showing a somewhat greater increase, but in the average period of observation of eight weeks the data are far from indicating full recovery to the conventional normal level.

A slight leucocytosis is another recognized feature of the condition, and the present data are in harmony with other reports. While the average count, ante-partum, falls inside the upper conventional boundary, many of the composing individual records exceed it, and the tendency is unmis-

TABLE 1

	TRIMESTER		
	1st	2nd	3rd
Hemoglobin	73.1%	69%	66%
Erythrocytes	4,050,000	3,950,000	3,880,000
Color index	0.90	0.87	0.85

TABLE 2

Blood morphology

	ANTE-PARTUM	POST-PARTUM	CONTROLS (ANTE-PARTUM)
Hemoglobin	66%	73%	72%
Erythrocytes	4,140,000	4,380,000	4,230,000
Color index	0.80	0.83	0.85
Leucocytes	9,680	7,910	9,100
Polymorphonuclear neutrophiles	69%	59%	68%
Lymphocytes	25%	34%	26%
Endothelial leucocytes	5%	5%	5%
Eosinophiles	1%	2%	1%

takable. Further, the increase in blood volume would tend to lower the actual concentration of the white cells, and if allowance be made for such a dilution effect, the leucocytes show a definite relative and absolute increase. The control group gives a consistent average. After delivery the leucocyte count shows a depression to values which more closely conform to the normal average.

The differential white cell count ante-partum is entirely normal. The basophiles are absorbed as the average is but a fraction of one per cent and rounded values are used for the averages. The control group show practically identical levels.

In a recent series of differential counts by Galloway (personal com-

munication) the average formula is neutrophiles 71 per cent, lymphocytes 23 per cent, endothelials 5 per cent, and eosinophiles 1 per cent. The agreement is most satisfactory.

The post-partum averages show a decline in the polymorphonuclear neutrophiles, with compensatory relative increase in the lymphocyte group. A slight upward tendency in the eosinophiles is also noted, though the average value remains within normal limits. Summarized, the only change in the differential count that these averages exhibit is from a leucoid blood ante-partum to a more lymphoid type after delivery. The differential count is, of course, uninfluenced by any change in the blood volume as this affects all components alike.

SUMMARY

The blood findings may be briefly summarized as follows:

1. Hemoglobin shows a relative and absolute depression of moderate degree during normal pregnancy.

2. The erythrocyte count is also lowered but by an amount of the order of the dilution effect from the hydremia which has been demonstrated to characterize the condition.

3. The two changes noted above determine a moderate secondary anemia with lowered color index.

4. The leucocyte count ante-partum shows a definite increase but one of moderate degree. The differential count is normal; the blood leucoid (adult) in type.

5. A tendency toward the restoration of normal levels characterizes the earlier months post-partum; an unique exception is recorded in the development of a relative lymphocytosis which depends largely on an absolute decrease in the polymorphonuclear neutrophilic elements.

The writer wishes to express his thanks to Drs. A. W. Burckel, C. E. Smith, W. L. O'Connor, and W. F. Donovan, for technical assistance.

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ELECTRICAL MEASUREMENTS OF NEUROMUSCULAR STATES DURING MENTAL ACTIVITIES

V. Variation of Specific Muscles Contracting During Imagination

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From the Physiological Laboratory of the University of Chicago

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In three of the foregoing studies (Jacobson, 1930a, b, d), on subjects previously trained to relax and on four subjects not trained, the instruction to imagine some activity involving the right arm is characteristically followed at once by evidences of contraction of muscle-fibers in that arm. But in one study (1930c), where instructions arouse visual imagination or recollection, evidence of the occurrence of eye-movements is characteristically detected. We are led to inquire whether the instruction to imagine bending the right arm or lifting a weight with that arm may not be followed by either contraction of muscle-fibers in that arm or visualization or by both.

The same question arises concerning a few tests, particularly on recollection of activities involving the right arm, where the results were negative for evidence of contraction in that arm, but where the subject suggested that he carried out the instruction through visualization alone. Accordingly, we need to test further the indications that during imagination or recollection, muscular contraction, if absent from one region, will be found in another.

The electrodes include all those previously described, but chiefly platinum-iridium needles. Leads from the biceps region continue as stated (Jacobson, 1930d). Where leads are from the ocular region, the point of one needle (+) is inserted through the skin inside the orbital ridge as close to the recti muscles as seems safe. The other needle is inserted to an approximately equal penetration in the ear-lobe on the same side.

In certain tests, as later indicated, where electrodes are applied in the ocular region, the lever is used simultaneously to record flexion of the right forearm. To signal the subject when to begin and to cease imagining, a telegraph key is sharply tapped (and at once released). When the click occurs, the circuit including the signal magnet is closed, so that the instant of the signal is recorded.

Results. In table 1 A are shown the results following the instruction to "visualize bending the right arm" when one electrode is attached over

TABLE 1

SUBJECT	INSTRUCTION	NUMBER OF TESTS	ELECTRICAL RECORD																ARM FLEXION					
			Results of tests			V°_{2m}						V'_{m}											Velocity	
						Microvolts during relaxation			Microvolts during test period			Microvolts during relaxation			Microvolts during test periods									
			+	−	?	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Present	Absent	Maximal extent average	Duration average	Cases constant	Per second
A. Visual imagination, electrodes in or over right biceps and coronoid fossa																								
																				mm.	*seconds*		*mm.*
B. E.	Visually imagine (a)	6	0	6	0	2	7	4	2	7	4	7	28	16	5	18	12						
B. E.	Control	1	1	0	0	0	2	1	23	41	32	7	28	16	12	12	12						
B. E.	Visually imagine (a)	2	0	2	0	2	7	4	2	7	4	7	9	8	9	9	9						
B. E.	Control	2	2	0	0	2	7	4	7	39	23	9	12	10	7	12	9						
B. R.	Visually imagine (a)	3	0	3	0	4	9	7	4	9	7	9	25	16	9	25	16						
D. M.	Visually imagine (a)	5	0	5	0	7	11	9	7	11	9	5	14	9	5	14	9						
D. M.	Visually imagine (a)	1	1	0	0	7	11	9	13	46	30	5	14	9	5	14	9						
D. M.	Control	1	1	0	0	7	11	9	13	27	20	5	14	9	5	14	9						
Total imaginings		17	1	16	0																		
Total controls		4	4	0	0																		
B. Visual imagination, electrodes over eye and ear-lobe																							
B. R.	Visually imagine (a)	2	1	1	0	9	17	13	17	42	30	13	21	17	13	42	22						
B. R.	Visually imagine (a)	1	0	1	0	9	17	13	9	17	13	12	35	23	12	35	23						
B. R.	Visually imagine (a)	3	3	0	0	13	30	18	9	30	19	12	35	18	69	69	69						
B. R.	Visually imagine (a)	3	3	0	0	9	17	13	9	21	15	8	84	42	34	84	63						

B. R.	Visually imagine (a)	6	6	0	0	13	21	17	13	42	17	8	76	25	21	105	55							
B. R.	Control 7	2	0	2	0	13	21	17	13	21	17	8	76	25	8	76	25							
B. L.	Visually imagine (a)	4	4	0	0	6	14	11	6	28	17	8	34	15	36	58	49							
B. L.	Control 7	1	0	1	0	6	14	11	6	14	11	8	34	15	8	34	15							
I. G.	Visually imagine (b)	6	6	0	0	6	24	12	6	24	12	12	48	30	84	276	180	0	6	0		0	0	0
I. G.	Control 7	1	0	1	0	6	24	12	6	24	12	12	48	30	12	48	30	0	1	0		0	0	0
I. G.	Visually imagine (a)	6	6	0	0	5	20	10	5	20	10	10	30	20	50	200	110	0	6	0		0	0	0
Total imaginings		31	29	2	0													0	12					
Total controls		4	0	4	0													0	1					

C. Imagination of flexing or lifting with the right arm, electrodes as in B

B. L.	Imagine (a)	6	0	6	0	3	12	6	3	12	6	24	132	78	24	132	78	6	0	0	37	5	4	6	0	07
B. L.	Control 7	2	0	2	0	3	12	6	3	12	6	24	132	78	24	132	78	0	2							
B. L.	Control 2	1	0	1	0	3	12	6	3	12	6	24	132	78	24	132	78	0	1							
B. L.	Imagine (b)	5	3	1	1	3	12	6	3	12	6	12	36	24	12	55	35	5	0	0	46	3	9	3	0	09
B. L.	Control 7	1	0	1	0	3	12	6	3	12	6	12	36	24	12	36	24	0	1							
B. E.	Imagine (a)	5	2	2	1	5	15	10	5	15	10	10	60	35	10	75	43	5	0	0	89	1	9	0		
B. E.	Imagine (b)	4	2	1	1	5	15	10	5	15	10	10	50	30	10	140	75	4	0	0	29	1	8	0		
B. E.	Control 7	3	0	3	0	5	15	10	5	15	10	10	50	30	10	50	30	0	3							
I. G.	Imagine (a)	7	2	4	1	4	25	8	8	59	25	4	13	8	4	101	34	7	0	0	11	4	4	0		
I. G.	Control 7	2	1	1	0	4	25	8	8	59	25	4	13	8	4	101	34	0	2							
I. G.	Imagine (b)	6	3	3	0	6	36	9	36	96	48	12	24	18	36	108	48	6	0	0	60	2	4	5	0	28
I. G.	Control 7	2	0	2	0	6	36	9	6	36	9	12	24	18	12	24	18	0	2							
Total imaginings		33	12	17	4													33	0							
Total controls		11	1	10	0													0	11							

Terms used in headings of columns are defined in previous articles. (a) = bending the right arm. (b) = lifting a ten pound weight with the right arm. Results are called "+" (col. 4) in A if the value in col. 12 exceeds that in col. 9; in B and C if the value in col. 18 exceeds that in col. 15. For summary, see text.

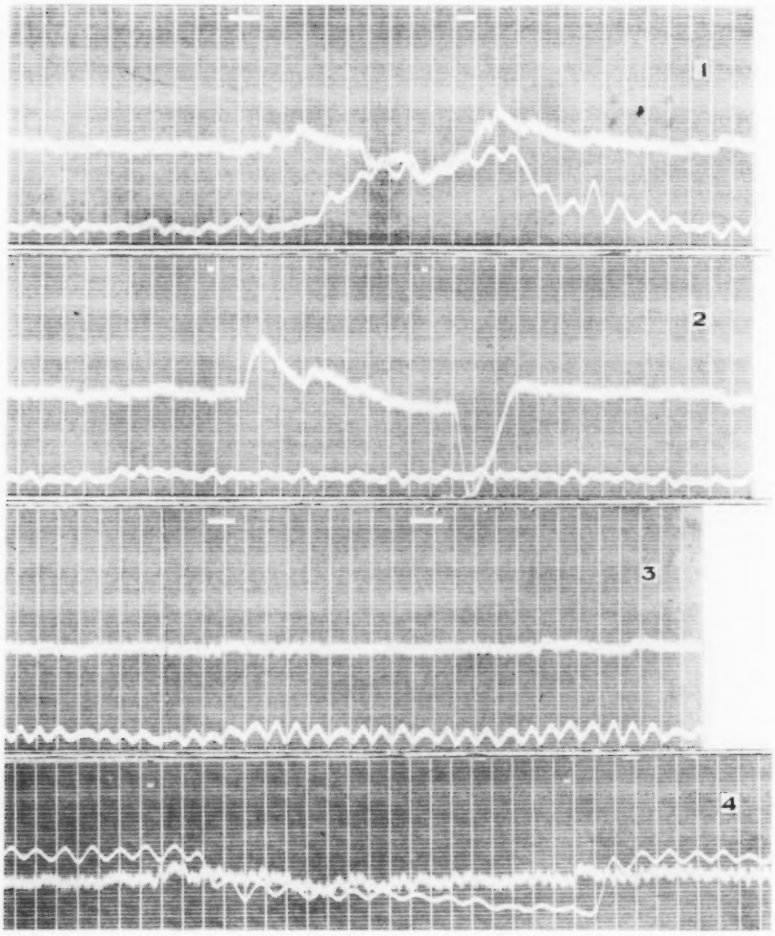

Fig. 1. The first signal (see top of photograph to the left) means, "Imagine bending your right arm," while the second signal means, "Relax any muscular tension present." Upper tracing is from the galvanometer string. One needle electrode, connected with the positive terminal of the amplifier, is inserted through the skin over the orbital ridge immediately to the right of the right eye-ball. The other needle electrode is inserted in the lobe of the right ear. Downward deflection indicates negative potential in the former electrode, 1 mm. = 10^{-5} volts. Lower tracing is from the shadow of the lever; upward excursion indicates right arm flexion × 40.

This photograph shows the presence during imagination both of e. m. f. arising from the ocular region (probably with eye-movement) and of arm flexion, amounting

the right biceps region and the other over the right coronoid fossa. Three subjects are employed who previously gave characteristically positive results in the form of action-potentials from the right biceps, when the instruction was merely, "Imagine bending the right arm."

Here, under the same conditions, excepting that the instruction is to visualize, action-potentials are absent from the arm-muscles in almost all instances (16 out of 17). That the conditions are really the same is shown in the control tests (interspersed in table 1, A) where the instruction is to "imagine muscularly." In these, action-potentials appear in characteristic manner.

On the other hand, when the electrodes are transposed to the ocular region, and the instruction is to "visually imagine bending the right arm," voltage changes V′m in the ocular region are recorded in almost all instances (29 out of 31, see table 1 B). That is, the subjects trained to relax respond differently to the two instructions, "Imagine bending the right arm" and "Visually imagine bending the right arm," the former response involving action-potentials from the right arm muscles and the latter response involving electrical variations from the contractions of the ocular muscles.

This interpretation harmonizes with the results of several special control tests interspersed among the foregoing with electrodes attached to the biceps region or with the lever attached to the right arm. When the subject is instructed "not to bother to visualize when the signal comes" (control test 7), no action-potentials are recorded from the ocular region.

A further set of tests is made with four subjects, three trained to relax and one (I. G.) not trained. In these, one electrode is in the ocular region and the other in the right ear-lobe, while the lever affords records of the movements of the right fore-arm. The instruction is to "imagine bending the right arm" or to "imagine lifting a ten-pound weight."

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to about $\frac{1}{2}$ mm. in 2 seconds. Velocity not here constant. Undulations in mechanogram are due to pulse movements. Vertical time lines $\frac{1}{5}$ second. Subject I. G., not trained to relax.

Fig. 2. Conditions the same as for figure 1, except that the instruction here is "Imagine seeing your right arm bending, slightly to your right. Visualize only." Here the electrical tracing quite resembles that secured from the same subject with the same set-up upon the instruction, "Look to the right." No flexion of the arm is here indicated.

Fig. 3. Conditions the same as for previous figures. The instruction is, "Do not bother to imagine." The results are negative.

Fig. 4. Instruction and general conditions the same as for figure 1, except that here the upper tracing is from the shadow of the lever and downward excursion indicates right arm flexion × 78. In this test, a flexion of practically constant velocity occurs, averaging about 0.07 mm. per second, while the electrical record from the eyes is practically negative. Needle electrode attached to positive terminal of amplifier inserted in the skin of eye-brow above the middle of the left eye. Other needle electrode inserted in lobe of left ear. Subject B. L., trained to relax.

Results may be considered separately for the various subjects (table 1 C). Subject B. L., in 11 tests, minutely flexes her fore-arm in all instances, but generally shows no potential differences V'_m between the electrodes on the ocular region and the ear-lobe (7 out of 11 instances). We may interpret that she responds to the instruction with minute arm movement, and with visualization in some but not in most instances.

Subject B. E. likewise minutely flexes his arm during imagination in all tests (9) but potential differences V'_m in the above mentioned electrodes occur in about half the instances.

Subject I. G. likewise minutely flexes his arm in all tests (13), but the results from the ocular region are positive for V'_m in 5 of the tests and for $V°_{2m}$ in 7 of the tests.

Regarding the results from platinum-iridium electrodes inserted in the ocular region and ear-lobe as above described, certain matters remain unsolved. Their point of application is closer to the recti muscles than is that of the platinum electrodes inserted in glass cells applied to the ocular region. The latter electrodes, with the present circuit, characteristically yield changes in V'_m rather than in $V°_{2m}$ during eye-movement; a fact which we presumed to be due to the relatively great distance between the electrodes and the recti muscles. Accordingly, the change to needle electrodes (inserted nearer the recti muscles) should augment the number of instances in which $V°_{2m}$ increases during eye-movement. This is what has seemed to occur, at least in the tests with I. G.

During the tests with I. G., he remarked that the imagined object was generally visualized directly in front of him. Upon applying the glass cells with inserted electrodes to the ocular region of this subject, he was given practice, with open eyes, in looking straight off in the distance and then converging, following a signal, upon a point about 20 cm. distant in the same line of vision. Presumably owing to insufficient amplification, we failed to get photographs showing electrical changes distinctive of convergence with the above-mentioned electrodes, and did not quite succeed even with the needle electrodes. To obviate this difficulty in recording, the instruction to this subject was worded "visually imagine slightly to your right," with the results as recorded in table 1 B.

Accordingly, the results with attachments simultaneously to the ocular region and to the right arm indicate that these subjects react to the instruction with movements of the right arm in all instances and with movements of the eyes in many but not all instances.

The subjects report that they imagine bending the right arm either through a muscular experience as of bending the right arm or through visual images of the arm performing the act. In response to the instruction "to imagine," they report that frequently they engage in both of the

above experiences. Our objective tests evidently harmonize with their subjective reports (cf. Galton, 1883).

CONCLUSIONS

1. Imagination of activity of the right arm (or other part) is characterized by contraction of muscle fibers either in that part or in the ocular region or in both localities.

2. This affords further evidence that mental activity is not confined to closed circuits within the brain, but that neuromuscular regions participate.

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ELECTRICAL MEASUREMENTS OF NEUROMUSCULAR STATES DURING MENTAL ACTIVITIES[1]

VI. A Note on Mental Activities Concerning an Amputated Limb

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Evidence has been presented that mental activities such as imagining or recalling bending the right arm or lifting a weight with that arm are characterized by the contraction of muscle-fibers in that arm (Jacobson, 1930 a, b, d, e). This leads us to inquire what occurs in individuals with amputated limbs who report that they can do everything in imagination with the lost part that they can do with the intact part.

The left arm of a graduate student at the University of Chicago had been amputated above the elbow joint at about the age of eight. He said that he could imagine doing anything with his left hand that he could do with his right. This assertion would harmonize with our results if it meant that upon imagining acts with his left hand he merely visualizes or verbalizes the action; but not if it meant that he could imagine acts with the lost hand fully in the same physiological manner as normal subjects.

The subject was a man of about 40. He presented a stump which included the atrophic remnant of the biceps-brachial muscle-group. The left humerus was about 29 cm. long, being 2 cm. shorter than the right. Its terminal four centimeters was covered chiefly by scar tissue, but above this level was some muscular tissue. Glass-cells containing plain platinum electrodes immersed in 0.9 per cent NaCl solution were employed with connections as previously described.

Preliminary tests were performed with one electrode attached over the flexor muscles of the right hand and the other on the skin over the right coronoid fossa to determine what reactions would follow the instruction to imagine bending this intact hand. Of 12 tests, 8 were positive for action-potentials signifying muscular contraction. Explanation of the negative tests is perhaps to be found in the subject's statement that he generally

[1] I wish to thank Miss Bernadine L. Lufkin for aid in preparing this and the foregoing manuscripts.

merely visualized bending his right hand. However, he showed greatly delayed reaction and relaxation times in these early tests, and the results should probably be considered as due to lack of practice in a tense subject in carrying out the instruction to imagine. Tests performed at a later date, with electrodes on the right biceps region, following the instruction to imagine bending the right arm, show action-potentials without unduly delayed reaction and relaxation times in all 5 tests made.

To determine what happens when this man imagines bending his missing left hand, tests are made chiefly of two types: A, with electrodes in the region of the partly amputated biceps muscle, and B, with electrodes in the region of the muscles which flex the right hand. The purpose is to

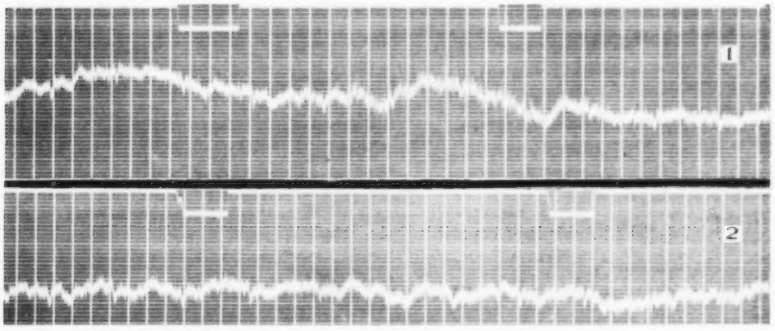

Fig. 1. The instruction is to imagine bending the left hand. One electrode (+) attached over remnant of biceps. Other electrode attached over non-muscular area near the tip of the stump. About 1.3 seconds after the first signal, action-potentials appear from the remnant muscles, ceasing about 0.4 second after the signal to relax. Downward deflection indicates negative potential in former electrode, 1 mm. = 2.3×10^{-5} volts. String not critically damped.

Fig. 2. Conditions the same. Control record, showing negative results following instruction to imagine bending the left foot.

test whether contraction occurs in either of these two regions during mental activities involving the missing hand.

A. *Electrodes over muscular stump and scar-tissue near the tip of the bone.* 1. Upon imagining bending the left hand, the stump biceps muscle generally shows action-potentials (13 out of 14 cases). Two control tests, following the instruction not to bother to imagine, prove negative. Likewise, upon imagining lifting a ten-pound weight on the fingertips of the left hand, the results are positive (4 tests), while control test 7 is again negative (figs. 1 and 2).

2. Additional control tests include the following: *a.* Upon imagining bending the left foot, the results are negative (9 tests), excepting the first

two tests. Possibly these two tests may be disregarded on the grounds of lack of practice following this particular instruction. *b.* Upon imagining bending the right foot, all tests (5) except the first prove negative. *c.* Upon imagining bending the right hand, results are positive in all tests (7). *d.* Upon imagining lifting a ten-pound weight in the right hand, the tests (7) prove positive in the first three and negative or doubtful in the last four.

The results recounted above indicate that the subject generally contracts fibers in the stump biceps muscle during mental activities concerning his missing left hand. Like the various normal subjects previously tested, following preliminary practice, imagined activities of other parts of the body not involving any part of the left arm are not accompanied by action-potentials in the muscles of that arm, excepting imagined activities in parts of the right arm. Here action-potentials occur in the stump of the left biceps-brachial group, suggesting that activities in the two arms are not so sharply dissociated in this individual as in normal subjects.

B. *Electrodes over muscles that flex the right hand and over coronoid fossa.* Following the instruction to imagine bending the left hand, all tests (6) show the presence of action-potentials in the above mentioned muscles.

It is evidenced that when this subject engages in mental activity concerning his left hand, certain muscles contract; but these, for example, in imagined flexion, instead of being merely the muscles that flex the left hand as in intact subjects, are in the stump of the upper arm or in the intact arm or in both places. The subject was not informed as to the purpose or methods of the investigation. It was therefore of striking interest when, after he had evidently engaged in subjective observation during a number of tests, he suddenly volunteered that he desired to correct his original statement that "he can imagine doing anything with his left hand that he does with his right." He now stated that when he does something with his right hand the left seems in imagination to duplicate the performance, going through the same experience. But he never has experiences of his left hand performing any act independently of the right. He adds, "*My imagination of bending the left hand is but a shadow,—a duplicate of what the right hand is imagined to perform.*" In short, his original statement is ambiguous and he corrects it. He can imagine doing anything with his left hand that his right hand does, but only under one condition: namely, that the right hand, at the moment of the imagination, actually engages in that same act or is imagined to engage in that same act. No independent imagination, such as exists for intact subjects, exists for this individual's left hand.

Supplementary tests were performed to compare this subject's reactions with normal subjects upon imagining bending the right and left forearms. Electrodes were attached in the right biceps region in the manner previ-

ously described (Jacobson, 1930a, b). Upon imagining bending the right arm, action-potentials from the right biceps region are clearly marked in all tests. Both reaction and relaxation times are prompt, evidencing the result of practice in following directions as compared with the earliest tests. Of 10 tests following the instruction to imagine bending the left fore-arm, none clearly shows action-potentials from the right biceps region. Generally after a much delayed reaction time (4–5 sec.) a very slight increase of V_{2m} is noted, followed by a much delayed relaxation time. Upon imagining bending the left hand, the results are predominantly negative (of 9 tests, 6 negative, 3 doubtful positive). Evidently, so far as the tests on imagined bending of the fore-arm are concerned, the subject, having at least a remnant of the muscles involved on either side, behaves like an inexperienced and rather tense normal individual, showing no skill at relaxation.

We find, then, that in this subject's mental activities involving the imagined or recalled use of the lost muscles which flex the left hand, a substitution-reaction occurs in the corresponding muscles of the right hand or in the remnant muscles of the left upper arm or in both. In normal subjects, it was found, at least after a little practice, that mental activities involving the muscles of the left hand generally are accompanied by no reaction in the right arm. A certain measure of independence exists for the two sides, particularly after a little practice. This independence is apparently lost for this subject with respect to the muscles controlling the hand. But the independence seems preserved for the upper arm muscles, excepting that this subject tends in greater measure than normal to have contractions in his left upper arm muscles associated with imagined activities involving his right hand.

CONCLUSIONS

1. Evidence is presented that in an individual who has suffered the total loss of a part by amputation, specific muscular imagination or recollection of movements in that part no longer takes place. After repeated observations, the subject reports that his imagination is "shadowy" for movements of the lost part.

2. During such mental activities, contractions (not characteristic in intact individuals) occur in other specific localities, evidently as substitutions. With these substitute contractions (and with visualization or verbalization such as may also occur in the intact), this crippled individual engages in mental activities involving the lost part.

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THE OXYGEN PULSE IN ATHLETIC GIRLS DURING REST AND EXERCISE

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Although numerous studies have been made of the metabolism of men in rest and exercise, and under various other conditions, not many such studies are as yet available in the case of women. The investigation here reported was made during April 1928, on a group of young girl athletes, through the courtesy of the late Director of the New Haven Normal School of Gymnastics. A series of observations was made on the oxygen consumption during rest and exercise, and this was correlated with the pulse rates.

METHOD. The apparatus was the same as that used by Henderson and Seibert in their aviation studies in 1918. The capacity of the spirometer was 100 liters. The two horizontal arms of iron pipe contained an inspiratory and an expiratory valve respectively. To the end of each arm was attached a light, wide bore, corrugated rubber tube 1 meter in length, the ends of which were connected to a mouthpiece with a trap for saliva. The exhaled carbon dioxide was absorbed in a carton of alkali. On the right of the tank was attached a centimeter scale on which slid a metal pointer fixed to the upper surface of the inner tank. One centimeter on this scale represented one liter of air in the spirometer; and the oxygen consumed was determined by reading the maximal point of the excursion, i.e., the end of expiration, at periods of one minute timed by stopwatch. The extent to which the spirometer fell in one minute gave the rate of oxygen consumption. The end of expiration was used in preference to the end of inspiration as representing a more constant level. About 15 liters of oxygen from a cylinder were added to 85 liters of air in the spirometer, so that the subject breathed a mixture of oxygen-rich air. Previous to each observation, the spirometer was emptied, ventilated, and refilled with fresh air and oxygen.

The subject wore a clip to close the nose, and neither it nor the mouthpiece was removed throughout the duration of the experiment, which lasted 12 to 15 minutes. Each experiment included:

1. A preliminary rest period of a minimum of 5 minutes standing at ease;
2. An exercise period of 2 minutes of vigorous standing running; and
3. A recovery period of a minimum of 5 minutes standing at ease.

In some cases the first period of the experiment, while the subject was standing at ease, was extended to 8 or 10 minutes. As a rule, however, it was found that 12 or 15 minutes appeared to be the optimum for the length of the experiment; for this length of time the subject was comfortable throughout and did not fidget or seem fatigued.

Although there was no attempt at measuring the amount of work done, there is no doubt that in every case the subject performed vigorous exercise. In two cases it was so vigorous that the subjects stopped at the end of one minute, but these data are not included. The girls ran well; the knees were lifted high; the pace was brisk, averaging 185 steps per minute; and the pulse rates were those of vigorous exercise, ranging from 120 to 168. The pulse rate was taken in the neck.

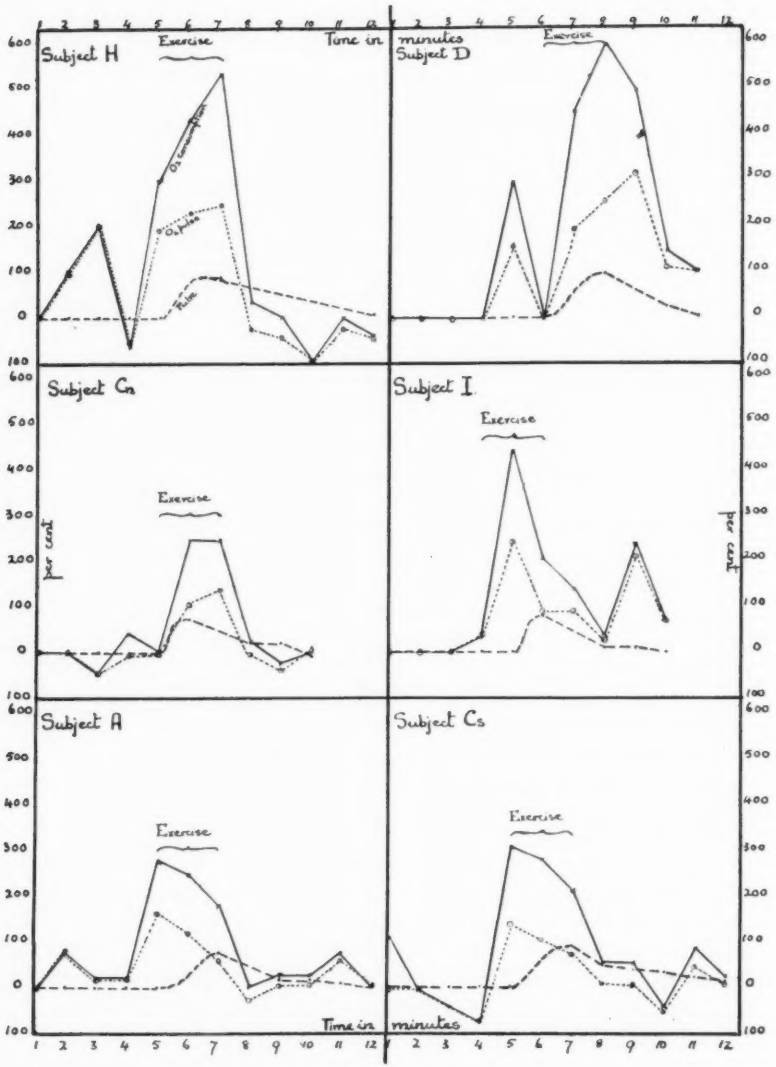
The clothes consisted of the usual lightweight gymnasium suit. The age variation was 18 to 22 years. For the calculation of the surface area from DuBois' tables, the heights were measured, and the weights (stripped) were taken from the latest records of the physical examinations. All the experiments were made in the mornings from 9 to 12:30.

The criterion used for the termination of the experiment after the third recovery period was the point at which the pulse rate had either returned to that observed at the beginning, or very nearly approached it.

Results. The results obtained are summarized in the accompanying graphs of 6 typical cases selected from 20 observations. The pulse rate, oxygen consumption and "oxygen pulse"—i.e., the amount of oxygen consumed by the body from the blood of one systolic discharge of the heart—are plotted against time. In order to make the comparisons as nearly on common ground as possible, the percentage increase or decrease above or below the resting level of oxygen consumption and oxygen pulse was calculated and is given in the graphs per square meter body surface per minute.

While the curves expressing the relation of the oxygen pulse to the heart rate are subject to minor irregularities, they tend towards a definite general form. The variations may in part be accounted for by the fact that many conditions other than the oxygen consumption influence the pulse rate. In some cases the heart rate was still accelerated after the exercise period even at the end of 5 minutes of recovery, when the oxygen consumption had either returned to the resting value, or very nearly approached it, e.g., in Cs an acceleration of 17 per cent; M, 8 per cent.

In practically every case there is some irregularity in the amount of oxygen consumed per minute preliminary to the exercise period and during recovery. The anticipation of the signal for the commencement of exercise is probably one factor contributing toward an increase in oxygen consumption before exercise. The subjects were at no time disconnected from the respiration apparatus until at least 5 minutes had elapsed following the



Figs. 1 to 6. Showing curves for the oxygen consumption (solid lines), pulse rate (broken lines) and the oxygen pulse (dotted lines). The variations are followed through a preliminary 5 minutes standing at ease, 2 minutes vigorous standing running and 5 minutes of recovery. The percentage increase or decrease above or below the initial resting level was calculated and is expressed in the graphs per square meter body surface.

work, so that the immediate rapidly changing values of oxygen consumption, ventilation and pulse rate in the transition from work to rest, emphasized especially by H. M. Smith (1922), were recorded.

Although this investigation was conducted with none of the elaborate detail of the study made by Smith on the physiological requirements for level and grade walking, it was found in agreement with the latter piece of work that while the larger part of the adjustment of the body to the demands of exercise in the transition from standing to running occurs very rapidly—within approximately half a minute—the recovery after exercise is not so prompt. The oxygen consumption fell continuously but did not reach the pre-exercise level until the end of the 5 minutes of recovery, and in some cases not even then. The use of a mouthpiece in experiments on breathing is not ideal, but is probably less of a disturbing element during the performance of heavy work than in rest. The error from this cause in these experiments is probably small. Smith found it did not exceed 2 per cent.

As only the one brief period of vigorous exercise was studied, no data can be given to show to what extent the oxygen pulse alters during slight and moderate exertion. The values for the oxygen pulse at resting rates of heart beat ranged from 2.8 to 5.5 ml., average 4.6 ml., or 0.044 to 0.084 ml. oxygen per kilogram body weight. This shows a close correspondence to the values obtained on men by Henderson and Prince (1914). It is of interest to note from the calculation of the oxygen pulse that approximately two to three times as much oxygen was used per systolic discharge of the heart during exercise as during rest. In general the values for the maximal oxygen pulse occurring at heart rates of 120 to 168 beats per minute lay between 9.0 to 16.6 milliliters per beat. The maximal oxygen pulse of 16.6 ml. was that obtained on subject M weighing 55.5 kgm.; on other subjects the body weights and corresponding maximum oxygen pulses were:

	SUBJECT							
	H	Cn	D	I	A	M	Cs	Cl
Weights in kilos	62	78	63	51.5	68.5	55.5	54	61.5
Maximum oxygen pulse	11.3	13	9.9	11.9	10.7	16.6	10.5	9.6

The oxygen pulse during recovery ranged from 1.2 to 11.1 ml. per beat at heart rates of 120 to 72 per minute.

For the sake of comparison, the oxygen pulse in university athletes is of interest; it has been calculated therefore from the data of 5 men, obtained by Henderson and Haggard in 1925.[1] The work done ranged from

[1] See table 8, page 246.

500 to 1500 kgm.-m. per minute on a bicycle ergometer. In all 5 cases from 2.0 to 3.6 times as much oxygen was used per heart beat during exercise as compared with rest.

	PULSE PER MINUTE	OXYGEN PULSE
		milliliters
A. N. W., 84 kilos:		
Standing at ease	62	6.50
650 kgm.-m. per minute	120	16.70
H. T. K., 87 kilos:		
Standing at ease	68	8.80
1500 kgm.-m. per minute	152	30.00
B. M. S., 80 kilos:		
Standing at ease	88	4.20
500 kgm.-m. per minute	136	8.85
F. A. G., 69 kilos:		
Sitting on bicycle	76	5.20
500 kgm.-m. per minute	120	14.80
M. C. H., 80 kilos:		
Sitting on bicycle	64	5.86
500 kgm.-m. per minute	125	11.95

In view of the fact that the amount of work done by the girls was not measured, the close correspondence of the oxygen pulse at heart rates of 120 to 168 to that of the men at similar pulse rates when doing 500 kgm.-m. per minute is of interest.

The oxygen pulse is one of the main factors determining the total energy which can be commanded by man during periods of stress, its maximum value depending on the hemoglobin index and the stroke volume of the heart. The possible relation between the oxygen pulse and the stroke volume of the heart is debatable, and one beset with obvious difficulties.

In considering the increase in the arterio-venous oxygen difference as one of the reserves possible in the circulation, suppose the muscles during the exercise period take up two-thirds of the oxygen of the arterial blood. If the hemoglobin index be 100 and the systolic discharge of the left ventricle 100 ml., the amount of oxygen in the arterial blood would be 18.5 ml., in the venous blood approximately 6 ml., the oxygen pulse being 12 ml. (Actually the majority of the subjects in this investigation had a mean maximal oxygen pulse of approximately 12 ml.) As it is unlikely that the utilization of oxygen by the tissues is ever complete, the venous blood even during severe exercise probably contains at least 2 to 3 volumes per cent oxygen or more. It would seem safe to assume therefore that at heart rates of 120 to 168 the volume output of the left ventricle per beat is at least 100 ml., or 1.6 ml. per kilo body weight. The volume of the systolic

discharge at resting heart rates for an oxygen consumption of 300 ml., or 4.8 ml. per kilo, an arterio venous oxygen difference of 5 volumes per cent, and a pulse rate of 70, would be about 86 ml., or 1.38 ml. per kilo. This would mean that the output per minute in a girl weighing 62 kilos would be about 6 liters as compared with 14 liters during work.

It thus appears that in subjects of good physique and in athletic training there is during exercise not only an increase in the pulse rate, but also in the output per beat. Evidently an accelerated pulse rate and abbreviation of diastole do not diminish the filling and stroke volume of the heart.

SIGNIFICANCE OF THE OXYGEN-PULSE. The oxygen-pulse is a somewhat tantalizing function. It is obtainable with precision, since both its factors, the pulse rate and the respiratory oxygen consumption of the body, are readily and accurately determinable. It brings us fairly close to a measurement of the output of the heart per beat, or stroke volume. Yet in the absence of one additional factor it leaves the stroke volume unrevealed. The additional factor needed is the arterio-venous oxygen difference, that is, the amount of oxygen that each unit volume of arterial blood loses before it returns to the lungs.

Very recently Baumann reports that he finds that puncture of the right heart in man may be done without injury. In the venous blood so obtained he finds 5.2 volumes per cent less oxygen than in blood taken from an artery simultaneously.

With this determination of the arterio-venous oxygen difference the oxygen pulse becomes a function of high importance and practical usefulness.

SUMMARY

In athletic girls the stroke volume is distinctly increased during exercise.

After 2 minutes of maximal or nearly maximal exercise, the pulse returns to the resting rate by the end of 5 minutes. The rate of oxygen consumption is also nearly that of the previous rest period within 5 minutes.

Two to three times as much oxygen is used per systolic discharge of the heart during exercise as compared with rest.

A close correspondence exists between the values for the oxygen pulse of the girls during the exercise and those of men at similar pulse rates when doing 500 kgm.-m. work per minute.

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THE RELATION BETWEEN ANOREXIA, ANHYDREMIA AND GASTRIC ATONY IN DOGS DEPRIVED OF WATER[1]

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Recent studies on vitamin B[3] deficient animals reveal the occurrence of 1, a decrease in water intake and anhydremia (Stucky and Rose, 1929; Sure and Smith, 1929) and 2, anorexia associated with a decrease in gastric motility (Rose, Stucky and Cowgill, 1930a). Are these two phenomena interrelated? If so, can such a relationship be demonstrated in the absence of B-avitaminosis?

In a previous communication it was reported that dogs, which received a ration qualitatively complete but restricted as regards the food and *water* intake to that consumed by vitamin B deficient companions, manifested a decrease in gastric motility similar to that shown by the B-deficient animals (Rose, Stucky and Cowgill, 1930b). These experiments make it apparent that a lack of vitamin B was not the sole factor affecting the stomach contractions.

A recent investigation (Rose, Stucky and Mendel, 1930) has shown that in B-avitaminosis food and water intake are probably related to each other. Perhaps the nearest approach to uncomplicated starvation and a study of its effect on gastric motility is to be found in the work of Carlson (1916) who states that " . . . . the decrease in the intensity of the hunger sensation was not due to a decrease in the intensity of the gastric hunger contractions. . . . . " This suggests that the partial starvation

[1] A preliminary report of these experiments may be found in the Proceedings of the Society for Experimental Biology and Medicine, xxv, p. 688, (May) 1928.

The data in this paper are taken from a dissertation submitted by William B. Rose in partial fulfilment of the requirement for the degree of Doctor of Philosophy, Yale University, 1928.

[2] Porter Fellow of the American Physiological Society, 1926–27.

The term vitamin B is used in this paper to refer to the mixture of food factors recently shown to occur in common natural sources of what has hitherto been called vitamin B (Smith, 1928). The commercial wheat germ product *vitavose*, obtained through the courtesy of E. R. Squibb and Sons, New York, was used as the source of vitamin B in these experiments.

which occurred in our control dogs exerted little or no influence on their hunger contractions.

In view of the above considerations the restriction of *fluid* intake assumes major importance as a possible cause of the decreased gastric motility observed in our control dogs. Therefore the effect of anhydremia, resulting from water deprivation, on the motility of the empty stomach of normal dogs was investigated.

METHODS. Comparative observations on appetite, blood concentration, and gastric motility were made before, during, and after a six-day period of water deprivation.

Diet. In studying a problem of this nature it is essential to use a ration having an almost constant water content. Accordingly, the casein diet III described by Cowgill (1923), supplemented by a potent source of vitamin B, was employed. The food was kept in tightly covered containers to minimize evaporation. The diet is complete in every known respect and its chemical composition is practically constant. It is conceivable that marked fluctuations in electrolyte intake might affect the fluid balance, one of the chief variables under consideration in this investigation.

Four dogs in good nutritive condition were kept under observation for at least a month prior to the period during which they were deprived of water. Each dog was given enough of the ration to maintain a practically constant body weight. During this preliminary observation period the animals consumed the daily allotment of food in less than a half-hour.

Blood. Numerous determinations of hemoglobin and whole blood chlorides were made from time to time. Occasional estimations of total blood solids served as a further check on blood concentration. The analytical methods used were the same as those employed in previous experiments (Stucky and Rose, 1929). Bulatao and Carlson (1924) showed that the blood sugar level bears some relationship to gastric motility. Hence, it appeared desirable to estimate the blood sugar in these dogs on this artificial diet. Abnormal values for any of these blood constituents were never encountered during the preliminary period.

Gastric motility. The technique employed for recording gastric motility has been described elsewhere (Rose, Stucky and Cowgill, 1930a). Data on the motor activity of the stomach of each dog were secured during the preliminary as well as the other periods of observation. Repeated tracings of gastric contractions taken for many hours at a time in the course of the preliminary control period revealed vigorous hunger contractions, excellent tonus changes, and physiological rhythm in every case. In some instances, continuous 24 hour records of the motility of the empty stomach were obtained.

EXPERIMENTS. Five experiments were performed with four dogs, one of the animals being subjected to two successive periods of water deprivation.

A brief summary of the data is presented in table 1. A typical case (dog 32) will be described in detail. Only a few of the more important facts in connection with the other experiments will be given.

Dog 32. On the first day of the water deprivation period this animal weighed 6.3 kilos and its hemoglobin was 96 per cent (Haldane). The dog

TABLE 1

Effects of water deprivation on blood concentration and gastric motility in dogs

DOG	PRELIMINARY PERIOD*		WATER DEPRIVATION PERIOD			RECOVERY PERIOD (WATER AD LIBITUM)		
	Gastric tracing	Hb	Day	Gastric tracing	Hb	Day	Gastric tracing	Hb
		per cent			*per cent*			*per cent*
32	Normal†	96	3rd	Shallow contractions	113	2nd	Shallow contractions	118
						6th	Vigorous contractions; normal sequence	90
			5th	Gastric atony	125			
			6th	Gastric atony				
33	Normal	108	4th	Minute contractions		1st	Moderate contractions	100
			6th	Shallow contractions	126	5th	Small contractions; good tonus	110
						12th	Vigorous contractions; normal	
34‡	Normal	100	4th	Slight variations in tonus		1st	Shallow contractions	96
			6th	Gastric atony	120	3rd	Moderate contractions	
						5th	Vigorous contractions	87
35	Normal	101	3rd	Very slight tonus changes	116	2nd	Small irregular contractions	114
			5th	Gastric atony	128	4th	Extremely vigorous contractions	
						6th	Essentially normal	108

* This period immediately preceded that of water deprivation.

† Signifies vigorous hunger contractions, good tonus and normal sequence of active and quiescent periods.

‡ This dog was subjected to a second period of water deprivation which gave results similar to those reported here.

was given its daily food requirement but water was withheld. A gastric tracing showed excellent hunger contractions, well sustained tonus, occasional gastric tetany, and normal sequence. Short excerpts taken from gastric records of this dog are depicted in chart 1.

Three days later the hemoglobin increased to 113 per cent. The dog

manifested complete anorexia and the gastric record showed a pronounced decrease in motility. Gastric tonus variations were present but only small hunger contractions were obtained. The normal rhythm was absent. By the 5th day of the experiment the hemoglobin had increased to 125 per cent; definite loss in body weight occurred and anorexia continued. A study of the motor function of the stomach at this time showed gastric atony. Hunger contractions were not seen in a six-hour tracing. The tracing was essentially a straight line, indented only because of respiratory movements. The regularity and uniformity of the respiratory movements appear to indicate that the animal was comfortable when the tracing was being taken.

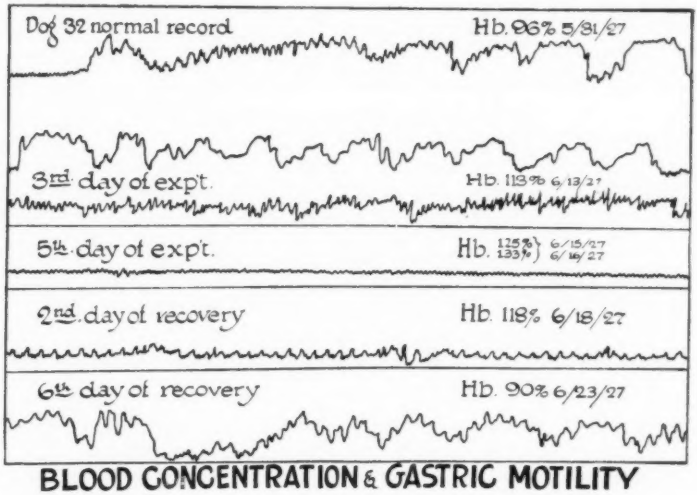

Fig. 1. Excerpts of gastric tracings obtained with dog 32, before, during and after a period of water deprivation. Approximately one-seventh original size.

The animal was then given water ad libitum. Food was always available. The dog drank with avidity but a few minutes later vomited most of what had been taken. It then drank more fluid but vomited again. However, after about a half-hour all of the fluid then ingested was retained. Later on in the same day some food (68 grams) was eaten, but complete anorexia was noted on the following day. This peculiar phenomenon was observed in other dogs of this series.

Forty-eight hours after fluids were allowed the hemoglobin decreased to 118 per cent. A gastric record revealed occasional shallow contractions and some variations in tonus. Subsequently the appetite improved, all the food offered was eaten, and the animal became more active.

Six days after water was first allowed the hunger contractions were vigorous, the sequence appeared normal and occasional evidence of gastric tetany was obtained. The hemoglobin was now 90 per cent. Five days later a continuous 24 hour record of the motility of the empty stomach failed to show any signs of abnormality.

Dog 34. This animal was one of the most active in the whole group. It had been used as a normal control in other experiments and its nutritive history for almost one year was known. Considerable data on its appetite gastric motility and blood concentration were available from the protocols of previous experiments. The gastric records obtained on this animal always revealed good contractions, marked tonus variations and physiological sequence. By the sixth day of water deprivation anorexia, anhydremia, and gastric atony were present although no other symptoms were in evidence. The animal recovered rapidly when water was allowed ad libitum. It was then given a complete rest for five days and again deprived of water. The results were essentially the same as those obtained during the first period of water deprivation.

Dog 35. The findings with this animal were similar to those from dog 32. However, the recovery was more rapid. Extremely vigorous hunger contractions appeared on the fourth day after unrestricted fluid intake. A gastric record obtained on the sixth day was apparently normal. On the tenth day a continuous twenty-four hour record revealed excellent contractions and sequence and was indistinguishable from a similar tracing taken in the preliminary observational period.

Discussion. In view of the rapidity with which anorexia, anhydremia and gastric atony developed in the animals employed in the current experiments, it is evident that serious physiologic disturbances may occur when dogs are deprived of water for short periods of time (six days). The average increase in hemoglobin value was only 22.3 per cent, yet gastric atony developed in *every* case. This is all the more significant because anhydremia is a systemic disorder, probably influencing the vital processes of every cell in the body. Inasmuch as the motor activity of the stomach is so markedly inhibited by moderate increases in blood concentration, it is not unlikely that the function of other muscles, particularly those of the involuntary system, may be affected.

Carlson (1916) has reported the occurrence of gastric atony in fever, a condition not infrequently associated with negative water balance. It is possible that a concomitant anhydremia may have been a contributing factor.

The early occurrence of anorexia in our dogs is not altogether surprising; but the rapid development of gastric atony more or less parallel with the loss of appetite is of interest. Vigorous motor activity of the empty stomach appears to be synchronous with the sensation of hunger (Cannon and Washburn, 1912; Carlson, 1912). One would, therefore, expect gastric atony to be accompanied by anorexia. The parallelism between the degree of dis-

turbance in gastric motility and the anhydremia that developed is also noteworthy. This suggests that the relation of blood concentration to gastric motility is more direct than that of diminished food intake; that the anhydremia rather than the anorexia may play the primary rôle in bringing about gastric atony. The value of these observations in furnishing a better insight into the triad—anhydremia, anorexia and gastric atony—that develops when animals are deprived of vitamin B, is discussed in another paper (Rose, Stucky and Cowgill, 1930b). None of the dogs employed in these experiments showed fever, vomiting (except when water was first administered) or other familiar symptoms of severe dehydration.

Tests of the contractile power of excised muscles, deprived of approximately 20 per cent of their normal water content, have been performed by Durig (1903). He failed to discover any impairment of function in such muscles. One is hardly justified in concluding that the same results would be obtained with intact muscles in which the blood and nerve supplies are undisturbed. Therefore, Durig's experiment cannot be accepted as evidence that muscles *in situ* continue to function with *optimum* efficiency when deprived of 20 per cent of their water content.

That the results of the current studies are probably not due to the influence of dehydration on the nervous system appears evident from the work of Thomas and Kuntz (1926), who demonstrated that gastric motility persists after section of the extrinsic nerves and elimination of the intrinsic nervous mechanism by nicotine poisoning.

There appears to be some relation between anorexia and a loss of hunger contractions. Complete anorexia was noted in practically all cases before gastric atony developed. On the other hand, the urge to eat was restored before the motility of the stomach became normal.

Certain writers have expressed the opinion that the body possesses a large factor of safety in the face of restricted fluid intake by virtue of a protective mechanism in the form of so-called water "reservoirs" which can be drawn upon when necessary. This function has been ascribed particularly to certain tissues, e.g., muscles, liver and skin (Engles, 1904; Skelton, 1927). No one doubts the fact that these tissues have a relatively large water content, but whether this high fluidity makes them reservoirs or not remains undetermined. The fact that these tissue units are prone to lose more water under the stress of dehydration than other organs does not prove that they act as physiologic water depots. It only demonstrates that these tissues may yield fluid without symptoms which are apparent from external appearance. Although one cannot say whether the gastric tissues of the animals used in the current investigation lost water during the period of fluid deprivation, it is clear that the motor function of the stomach was markedly impaired. It remains for future work to unfold the possible deleterious effects, in other tissues, of relatively small changes in concentration of the fluid which bathes them.

SUMMARY-CONCLUSIONS

Dogs in good nutritive state were fed an artificial ration complete in every known respect. Their appetite for this diet was excellent; repeated gastric tracings showed vigorous hunger contractions, good tonus variations and rhythm. Blood analyses revealed normal values for sugar, hemoglobin, total solids and chlorides. The animals were then deprived of water and the relationships between appetite, anhydremia and gastric motility were studied.

A moderate increase in blood concentration, evidenced by an increase in hemoglobin value of less than 20 per cent, was associated with a definite decrease in the motor activity of the empty stomach and partial or complete loss of appetite. A greater increase in blood concentration resulted in gastric atony in every case.

In one instance, when characteristic hunger contractions were absent but some tonus changes persisted, the animal ate all food offered. In the other cases, however, where atony of the stomach was recorded, there was a complete loss of appetite.

In view of the fact that anhydremia is a systemic disturbance it is suggested that other organs, particularly muscles, may fail to function with *optimum* efficiency in the presence of a moderate degree of anhydremia. In this sense, the results of the present study fail to support the contention that the organism possesses a great factor of safety with respect to fluid balance when it is deprived of water.

The authors are indebted to Mr. Alfred Weller for valuable technical assistance.

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DIET IN RELATION TO REPRODUCTION AND LACTATION. III

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Guest, Nelson, Parks and Fulmer (1926) in studies of certain grains as the sole source of vitamin B observed that a normal rate of growth was obtained with wheat, rye, barley and white and yellow corn. Reproduction was also normal, but there was a high mortality of the young due to abnormal lactation. It was also observed that a large number of females died during pregnancy or parturition on the diets employed in these experiments, and that the mortality varied with the kind of grain used as a source of vitamin B. The statement was made that the amount of vitamin B necessary for normal lactation is much greater than that required for normal growth and reproduction, and that the amount of vitamin B required for reproduction is not much greater than that required for normal growth. Since the above work was done it is now fully recognized that what was formerly designated vitamin B is composed of at least two factors, namely, the antineuritic and pp factors or, as they are also called, vitamins B and G respectively.

Daniels and Hutton (1925) have shown that successful reproduction and lactation may be obtained on a milk and soy bean diet and attribute this to the peculiar type of inorganic constituents in the ash of soy bean. When soy bean ash was added to whole milk, the diet gave favorable results on reproduction and lactation; whereas milk alone without soy beans or ash from soy bean failed in reproduction.

Although we know in a fairly satisfactory way the dietary factors necessary for normal growth and reproduction, little is known concerning the equally essential relationship between diet and lactation. This is of practical importance because failure in lactation manifests itself in live stock feeding. The failure of lactation in human beings is too well known to require comment. We have been interested for some time in the value of individual foods as a source of vitamins B and G for lactation when used singly and in combination. The object has been to gather data so that a more complete analysis might be made of complex rations from a lactation standpoint, since it is but a simple step from the single foods to the more complex diets.

The present communication deals with the effect of soy beans and various

animal organs on reproduction and lactation. Three varieties of soy beans (glycine hispida) were used, namely, Manchu, Virginia, and Peking or Sable. The Manchu is the yellow variety while the Virginia is brown; and the Sable bean is black. Ten, 20, 40 and 73.3 per cent levels of each soy bean were used in the experimental rations. The rations consisted of casein 18.0 per cent, salt mixture 185 (McCollum and Davis 1914), 3.7 per cent, filtered butter fat 5.0 per cent and various levels of soy beans; the balance of the ration to 100 per cent was composed of dextrin. The only source, therefore, of vitamins B and G was soy bean. The casein, butter fat, and dextrin were prepared according to directions given in an earlier paper(Guest, Nelson, Parks and Fulmer, 1926). The soy beans were cooked by steam for three hours at atmospheric pressure and subsequently dried and ground.

The results with soy beans were similar to those reported by Guest, Nelson, Parks and Fulmer (1926), on wheat, rye, barley, and white and yellow corn. The growth curves of all lots on the different levels of the various varieties of soy beans were normal. No appreciable differences were manifested as far as growth is concerned on the different levels of the three varieties of soy bean. Ten per cent of the three kinds of soy bean gave normal growth. It was therefore believed possible that higher levels would improve the rate of growth, but such was not the case. The mortality of the females was low on all the rations except in the case of the lot which received 10 per cent of Virginia soy bean as the sole source of vitamins B and G. The cause of the high mortality of the females in this lot is not known, but the mortality of most of the females in these experiments was not due to the same cause as that ascribed by Guest, Nelson, Parks and Fulmer (1926). The latter investigators found the vast majority of the females which died succumbed during pregnancy or lactation. Only three of the females on all of the levels of soy beans died in this condition. Thirteen females and eight males died of unknown cause.

Reproduction was good on several of the levels of the different soy beans employed. Only three females receiving 73.3 per cent of Manchu soy beans, and the same number of females on the 73.3 per cent level of Sable soy bean had more than one litter. These six females had 2, 6, 5, 2, 3 and 4 litters respectively. The 10 per cent level of Virginia soy bean gave similar results in that on this level also only 3 females produced more than one litter. These three females had 2, 6 and 3 litters respectively. Only two females from each lot receiving 40 and 73.3 per cent of Virginia soy bean had more than one litter, and reproduction on the other two levels was not exceptionally good. These four females had 4, 5, 2 and 6 litters respectively. On 40 per cent of Sable bean three females had no litters and the remaining three had 2, 3 and 4 litters each. Five or more females on each of the other lots of the different varieties of soy beans produced

more than one litter. The litters varied from two to nine for each female. Sufficient data have not been obtained to explain the decrease in reproduction on the higher levels of soy bean—especially the results obtained on the 73.3 per cent levels of all the beans and on the 40.0 per cent levels of Virginia and Sable. Reproduction was good on the 10, 20 and 40 per cent levels of Manchu soy beans. Only 2 females of 18 failed to reproduce on these levels, and 12 of the total number produced from 5 to 8 litters of young. Reproduction was also good on 10 and 20 per cent levels of Sable soy bean. Only one female of the 13 did not reproduce and 10 of the 13 had 5 to 9 litters. Three females on 20 per cent of Virginia soy bean had 3, 3 and 4 litters respectively. As a general rule six females and three males were placed in each lot, although the rations containing 20 per cent of Manchu soy bean, 10 per cent of Sable soy bean, and 10, 20 and 73.3 per cent of Virginia bean had 7, 7, 10, 7 and 7 females per lot. Four males were placed on the 20 per cent Sable and 10 per cent Virginia soy bean rations. The experiments were continued for about 325 days.

The per cent mortality of the nursing young decreased as the per cent of soy bean in the ration increased, as would be expected. Manchu soy bean seemed to give somewhat better results on the rearing of the young than the other two varieties of soy bean. Ten and 20 per cent levels of Sable bean and 10 per cent of Virginia bean gave the poorest results on rearing of the young. The mortality on all the rations varied from 25 to 100 per cent. The mortality of the young on 10, 20, 40 and 73.3 per cent levels of Manchu bean was 60, 50, 31 and 43 per cent respectively; whereas, on the same levels of Sable soy bean the mortality of the young was 100, 100, 72 and 25 per cent. The per cent mortality of the young on the corresponding levels of Virginia bean was 100, 83, 71 and 33. The average weight of the young when weaned was below normal in all cases except possibly in one litter of five, which was reared on the 20 per cent level of Virginia soy bean. The weaning weight of the young varied between 24 and 29 grams for most of the lots. The 20 and 40 per cent levels of Virginia soy bean gave weaning weights of 40 and 36 grams respectively; whereas 73.3 per cent of Sable bean in the ration resulted in an average weight of 33 grams for the young when weaned. Increase in the level of the soy beans did not appear to have any appreciable effect on the weaning weight of the young. Not more than six young were allowed each female.

The data on soy beans show that growth is normal on all levels, reproduction is normal on the lower levels, but lactation is decidedly abnormal on all the levels of the three varieties of soy beans employed. Many healthy young are born, but the mortality of the young is high; and the young that are weaned are decidedly below the normal weight at 30 days of age. The problem that presented itself was therefore to find out why such is the case and what individual food, if any, can be added to a soy bean diet so as to

make lactation normal and thereby increase the weight of the young when weaned at 30 days of age.

Various organs were therefore used as a supplement to a soy bean ration that has proved inadequate for lactation. The following organs were employed: lung, brain, thymus, liver, kidney, spleen, pancreas, and heart either from hogs or cattle. The basal ration consisted of casein 18.0, salt mixture 185, 3.7, soy bean 15.0, butter fat 5.0, and the remainder of the ration to 100 per cent was composed of dextrin. The casein, dextrin, and butter fat were prepared or purified as reported in an earlier communication by Guest, Nelson, Parks and Fulmer (1926). The various organs were incorporated in the basal ration in such a manner so as to replace an equivalent amount of dextrin. Rats were used in all of the experiments. Pregnant females were placed on the individual diets about five days prior to delivery. Unpublished data from this laboratory have shown that a diet adequate for normal growth and reproduction but not for lactation gives the same results as far as lactation is concerned whether fed to the recently weaned animal and continued through growth or to a pregnant female taken from a good diet. Diets that affect lactation adversely result in a high mortality of the young and the young are decidedly underweight when weaned. Whatever the factor or factors involved in lactation on these diets, the substance or substances are not stored.

The data are given in the table and they are for the most part self explanatory. The per cent mortality of the young from mothers receiving the different organs was for hog lung, 94, for hog spleen, 71, for hog pancreas, 48, and for beef thymus, 58. The average weights of the young when weaned from mothers receiving the same organs were 22, 31, 45 and 26 grams respectively. Practically the same results were obtained on pancreas whether from cattle or hogs. The average weight of young when weaned from mothers on beef pancreas was slightly lower (40 grams), and the per cent mortality was also somewhat lower (40 per cent). Brains from both cattle and hogs did not give quite as good results as pancreas—especially so from the standpoint of the weaning weight of the young. The per cent mortality on hog brain was 43, whereas on beef brain it was 26; and the weaning weights were 32 and 33 grams respectively. Beef heart gave a mortality of 73 per cent and a weaning weight of 42 grams.

The best results in this series of experiments were obtained on diets supplemented with 25 per cent of either hog kidney or beef liver, and on the ration containing 30 per cent of hog liver. The mortality of the young from mothers on two of these products was low, and the average weaning weights were far above those obtained when other organs were used. The mortalities were for hog liver 3 per cent, for beef liver 28 per cent, and for hog kidney 14 per cent; while the weaning weights were 68 grams, 61 grams and 63 grams respectively. One of the males whose mother received 15

per cent of soy bean supplemented by 30 per cent of liver from hogs weighed 90 grams when weaned at 30 days of age. The control for all of the experiments thus far mentioned, namely, diet number 68, contained 15 per cent of Virginia soy bean as the sole source of vitamins B and G. The mortality of the young on this diet was 88 per cent, and the average weaning weight

TABLE 1

Lactation on various diets

RATION NUMBER	ORGANS	NUMBER OF LITTERS	NUMBER OF YOUNG	DIED 0 TO 7 DAYS	DIED 8 TO 14 DAYS	DIED 15 TO 30 DAYS	PER CENT MORTALITY AFTER 7 DAYS	NUMBER WEANED	AVERAGE WEIGHT
68	0	8	48	15	0	29	88	4	22
70	30 H, lung	8	48	14	13	19	94	2	22
71	30 H, spleen	8	48	0	0	34	71	14	31
72	25 H, pancreas	8	48	6	0	20	48	22	45
85	25 B, pancreas	8	48	0	5	14	40	29	40
74	25 B, thymus	8	48	0	6	22	58	20	26
76	25 H, brain	9	54	1	9	14	43	30	32
87	25 B, brain	8	48	6	1	10	26	31	33
75	25 H, kidney	9	54	12	4	2	14	36	63
78	25 B, heart	9	54	6	15	20	73	13	42
77	25 B, liver	9	54	4	12	2	28	36	61
79	25 H, liver	10	60	17	8	3	26	32	67
81	5 H, liver	8	48	6	0	21	50	21	38
80	15 H, liver	10	60	9	17	3	39	31	61
73	30 H, liver	7	42	12	0	1	3	29	68
99	5 H, liver	6	36	0	0	36	100	0	0
82	15 H, liver	9	54	11	12	11	53	20	55
84	25 H, liver	8	48	0	7	8	31	33	55
83	40 H, liver	7	42	0	1	4	12	37	66
88	H, dried liver	5	30	0	12	18	100	0	0
93	H, dried liver	8	48	1	3	13	34	31	45
100	H, extracted liver plus extract	6	36	0	6	12	50	18	40
101	H, extracted liver	4	24	2	0	14	64	8	40
102	H, liver extract	5	30	6	0	17	71	7	18

was 22 grams. The soy beans were cooked by steam for three hours at atmospheric pressure. In view of the excellent results obtained with liver from hogs when supplementing soy beans as a source of vitamins B and G, it was deemed advisable to ascertain what effect would result with different levels of hog liver as the only source of these two vitamins. The

rations consisted of casein 18 per cent, filtered butter fat 5 per cent, salt mixture 185, 3.7 per cent, various levels of hog liver, and dextrin to total 100 per cent. These diets are numbered 99, 82, 84 and 83 in the table. The mortality of the young on 5 per cent of hog liver was 100 per cent, 53 per cent on the 15 per cent level, 31 per cent on the 25 per cent level; whereas a mortality of only 12 per cent was shown on the 40 per cent level. The average weaning weights were 55 grams for the 15 per cent level of hog liver, 55 grams for the 25 per cent level, and 66 grams for the 40 per cent level.

Diet 88 shows the effect of heat on liver in so far as lactation is concerned. Hog liver was dried in an electric oven for 12 hours at 120° C. The dried liver together with soy bean as the only sources of vitamins B and G was incorporated in ration 88. This diet contained dried liver equivalent to the solids of 25 per cent of the fresh material—replacing the 25 per cent of fresh liver of ration 79, so that the solids of rations 88 and 79 were the same. The mortality of the 30 young born on this ration was 100 per cent. Another batch of hog liver was dried over a steam plate by means of a current of air from an electric fan which kept a continuous circulation both above and below the pan, since the pan containing the liver did not come in direct contact with the steam plate. The product after drying and grinding was of a chocolate brown color. The per cent mortality of the rats from mothers on this ration (no. 93) was 34 per cent, and the average weaning weight at 30 days was 45 grams. The dried liver was incorporated in ration 93 in the same manner as in ration 88. It is evident that the lactation factor was destroyed by heating the liver to the higher temperature, as shown by the results obtained with diet 88. Results on ration 93 when compared to the data obtained on diet 79 show that considerable destruction had taken place, but the lower temperature was not sufficient to destroy all of the factor or factors by any means.

Rations 100, 101 and 102 show the effect of extracted liver and the extract from liver on lactation. The dried liver as prepared for animals on diet 93 was extracted with anhydrous diethyl ether. The extracted liver was prepared as follows: 500 gram lots of dried liver were placed in a flask and refluxed 24 hours with 800 cc. of anhydrous ether. The ether was decanted through a filter and the residue washed with 300 cc. of fresh ether. The process of refluxing and washing was repeated three times. The residue was warmed over a steam bath to remove any residual ether, and the dried product so obtained represented about 83 per cent of the original dried liver. The filtrates and washings from the preparation of the extracted liver were combined, and the ether removed by distillation. The residue—a heavy, almost black, viscous substance—constituted about 16 per cent of the original material. The mothers on ration 100 received 15 per cent of soy bean together with extracted liver and extract equivalent

to 30 per cent of the fresh liver and made up as for rations 88 and 93. The animals on ration 101 received extracted liver and soy bean. The extracted liver replaced the dried liver of ration 93. The rats on diet 102 received liver extract, replacing the dried liver of ration 93.

The mortalities on rations 100, 101 and 102 were 50, 64 and 71 per cent; and the average weaning weights were 40, 40 and 18 grams respectively. The mortalities were higher than on ration 93, and the weaning weights were lower. This might be expected for rations 101 and 102, but why it should be so for ration 100 is difficult to explain, excepting the possibility that some destruction of the lactating factor might have occurred in the course of treatment of the liver. It appears from the results on extracted liver that by far the larger amount of lactating material resides in the residue remaining after ether extraction.

The per cent mortality in all of the experiments was calculated on the basis of those surviving after the seventh day. All rations except 99, 82, 84 and 83 contained 15 per cent of cooked Virginia soy bean. In the table, H designates from hogs and B from cattle.

SUMMARY

1. Rats grow at a normal rate with 10, 20, 40 and 73.3 per cent of soy bean as the only source of vitamins B and G.

2. Reproduction was normal on the lower levels of soy bean, but on the higher levels the rats were not as prolific as on the lower levels.

3. Satisfactory lactation was not obtained on any level of soy bean investigated.

4. Lung and spleen from hogs and beef thymus did not supplement a 15 per cent level of Virginia soy bean so as to improve lactation appreciably.

5. Hog pancreas and pancreas, brain, and heart from cattle, supplemented the soy bean ration so an improvement in lactation was apparent.

6. Liver from hogs and cattle and hog kidney supplemented the soy bean ration in such a way that very marked improvement in lactation resulted. The young showed a superior rate of growth.

7. The lactating factor was destroyed in liver at 120° C.

8. Ether extract of liver possesses no appreciable lactating properties, but the residue does.

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EXPERIMENTS IN CROSS CIRCULATION

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These experiments were planned with the object of finding out how long it is possible to cross the circulations of dogs and also to ascertain what are the effects of a continuous exchange of blood between two animals. Methods for temporarily crossing the blood streams of dogs, cats, and rabbits had been described before, but none of the workers in this field had succeeded in maintaining the interchange of blood for more than a few hours. With the methods described by Bazett and Quinby (1), Anrep (2), and Heymans and Heymans (3) the animals had to be sacrificed at the termination of the experiment. No one has recorded crossing the circulations in unanesthetized animals. With the method described below it has been possible to maintain the continuous exchange of blood between normal unanesthetized dogs for five and six days.

A careful selection of animals is essential to the success of these experiments. Healthy adult dogs of equal size, each weighing between 12 and 15 kilos, are the ones of choice. It is preferable but not imperative to have animals of the same sex. It is most important to choose quiet friendly animals. The dogs making a pair do not have to be of the same breed. Mongrels with a large proportion of hound ancestry are particularly good.

The operative technic is as follows: The animals are anesthetized with ether after having had about two-thirds of their necks shaved and these areas thoroughly washed with soap and rinsed with ether. The dogs are then placed on their backs and fastened so that their heads are in contact. A rolled-up towel is placed under the cervical vertebrae to assure adequate exposure, and the operative fields are prepared with an acetone-alcohol solution of mercurochrome. The incisions are made parallel, 3 to 5 cm. in length, just posterior to the right external jugular vein of one dog and the left external jugular vein of the other. The posterior margins of the wounds are sutured together to block out the surrounding skin (plate I, fig. 1). The anterior portions of the wounds are redraped with sterile towels to exclude the possibility of skin contamination. The external jugular veins are dissected out for a distance of 4 to 5 cm. All tributaries are divided and ligated with silk (plate I, fig. 2). The carotid arteries are similarly mobilized. The temporary suture uniting the posterior margins

of the skin wounds is removed and the approximation of these edges is secured by carefully placed subcuticular sutures of silk. An effort is made to obtain perfect apposition with the minimal amount of tension. This requires a double layer of buried sutures. The mobilized vessels are then brought into view and occluded with rubber-shod clamps (plate I, fig. 3). These four vessels are divided as far cephalad as possible. Their distal ends are transfixed with medium silk sutures, and the proximal ends are carefully freed of superfluous adventitia.

The lumen of each vessel is thoroughly washed with warm salt solution and filled with sterile oil. The artery of each dog is united to the vein of

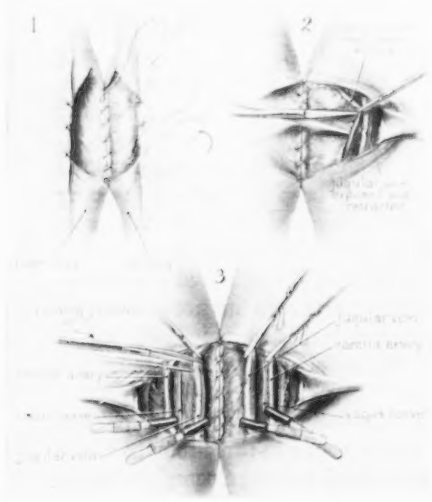

Plate I

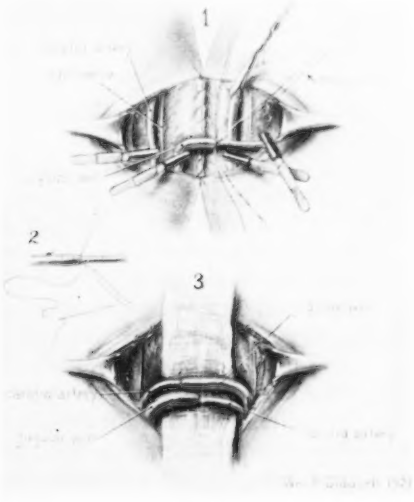

Plate II

the other by an end-to-end anastomosis, following Carrel's technic (plate II, figs. 1 and 2). The rubber-shod clamps are removed as soon as the vessels are joined together (plate II, fig. 3). If bleeding between the sutures occurs, it is controlled by gentle pressure with moist gauze. The closure of the ventral margins of the wounds is effected with the same degree of care and the same type of sutures that were used for the dorsal margins. No external cutaneous sutures are necessary, although in some cases a running epithelial suture assures perfect apposition. The air within the wound is aspirated, thus obliterating the dead space about the vessels.

A dressing of dry gauze is placed around the incision and between the

opposing skin surfaces. Tension is taken off of the line of closure by applying a broad band of adhesive plaster around the necks of the two dogs. It is necessary to avoid drawing this band too tightly. The animals are held together by one four-inch strip of adhesive plaster encircling their bodies two or three times at the level of the lower ribs. This is placed so as to allow a distance of from 3 to 4 inches between the dogs. The adjacent forelegs are also held together with adhesive strips. These coaptation bands have to be renewed from time to time. The dressing requires changing at least every 24 hours. When doing this it is important not to remove the band of adhesive around the dogs' necks, in order to avoid the possibility of their pulling apart.

In feeding the animals it is necessary for an attendant to be present to keep them from fighting. Experience has shown that dogs while bound together sometimes have difficulty in swallowing large morsels of food; consequently it is preferable to give them a diet of ground meat and bread. Whenever it is possible the dogs are kept out of doors and are given the range of the entire yard.

The crossing of the circulations between dogs that have been prepared according to the procedure just described has been maintained in many cases for 3 and 4 days and in two pairs for 5 days and in another two pairs for a little more than 6 days. During this period the animals ate, drank, and slept in a normal manner. They defecated and urinated when exercised. Their moving around was moderately restricted but not sufficiently so to prevent their walking and in a few instances running. Several of the pairs did not give evidence of any discomfort, annoyance, or fatigue throughout the course of the experiment.

The termination of the crossing of the circulations in over forty pairs of dogs came from a variety of causes. The outcome in three-fourths of the cases, however, was a thrombosis of the vessels following a complete breaking down of the wounds. This latter process was initiated usually by the wounds becoming moist and the skin edges separating slightly. Twenty-four to 48 hours later there would be wide gaping of the wounds, with exposure of the pulsating vessels, which, however, would not become occluded by thrombus formation until another 24 to 36 hours had elapsed. When the thrombosis in the vein of one dog began earlier or progressed more rapidly than that in the vein of his partner, the dog whose vein became occluded first usually bled to death into his mate. Two pairs pulled apart while the wounds were being dressed. In one instance the animals developed hematuria on the 5th day and died a few hours later. At autopsy the kidneys were large, swollen, and hemorrhagic. On two occasions one of a pair developed pneumonia and died. The partners, however, continued to live. In four instances one animal seemed to bleed to death into his mate shortly after the exchange of blood began. This was occasioned

by a constriction of the external jugular vein of the dead dog. In one pair as will be described below, death may have been due to an incompatibility of the cells and sera.

Among the first observations made during these experiments was that the pulsations of the anastomosed veins differ markedly from those in normal veins. Furthermore, as long as the arteriovenous communication is patent, one can detect a thrill and bruit over the anastomosed vessels. After the vessels are joined and the circulations first begin to cross, it is frequently observed that the blood pressure[1] of one animal rises 10 to 15 mgm. of mercury, whereas that of his mate falls approximately the same amount. After a period of time varying from 5 to 10 minutes the pressures begin to return slowly to their previous levels and tend to maintain these throughout the duration of the experiment. The blood pressures of the two dogs do not become equal, as is evidenced by the following observation. The figures indicate milligrams of mercury.

DOG	BEFORE OPERATION	10 MINUTES AFTER CROSSING	1 HOUR AFTER CROSSING	24 HOURS AFTER CROSSING
A	128	100	94	68
B	100	146	80	140

This fact was confirmed by us in ten pairs of animals. It tends to refute the general law formulated by Chabrol (4) that "toute hypertension chez l'un des animaux suscite une hypotension chez l'autre." Chabrol's law, however, is probably valid for sudden changes lasting for a few minutes. Nervous stimuli which do not evoke the secretion of a chemical affecting the intravascular tension cause reflex changes in the blood pressure of the stimulated animal only. Drugs such as epinephrin and histamin, injected intravenously into one animal, cause similar and almost synchronous changes in the pressure of each member of the pair. The differences in the time and the degree of the reactions are due to dilution of the drug and to the fact that it must pass through two pulmonary circulations before affecting the second dog. These observations do not hold, however, when one establishes a cross circulation by means of carotid-carotid and jugular-jugular anastomoses, for with this type of union the bloods are not mixed until after they pass through the cerebral circulation of one of the dogs. A drug, therefore, having different effects when injected into the cerebral or the peripheral circulation will register these differences in the members of a pair that are cross-circulated by this latter method.

We were able by such a preparation to confirm the observation of Anrep and Starling (5) upon the cerebral effect of epinephrin. We injected 0.5

[1] All blood pressure determinations were made by cannulating the femoral artery.

cc. of this drug into the femoral vein of dog A whose left carotid was anastomosed with the peripheral end of dog B's right carotid. A's pressure rose from 120 mm. of mercury to 210 mm. and then dropped to 104 mm., whereas B's pressure fell to 58 mm. from 118 mm. and then returned to 120 mm.

That the temperature of an animal is not controlled entirely through the blood is shown by the fact that the rectal temperatures of dogs whose circulations are crossing are frequently different. Hédon (6), (7) was the first to make this observation. Our experiences go to show, however, that the temperatures of these animals tend to have the same fluctuations. This is due probably to both dogs being subjected to the same external conditions. In one experiment we chilled one member of a pair by keeping the skin wet and exposing the body to the draught of air from an electric fan. This dog's rectal temperature fell 3.6°C. in one hour, whereas the rectal temperature of his mate rose 0.7°C. The blood pressures of both animals were taken continuously throughout the experiment. It is interesting to note that the effect of chilling was a very gradual rise of 20 mm. of mercury in blood pressure, followed by an equally gradual return to normal. The control animal that was not chilled failed to show the slightest alterations in blood pressure.

Studies of the blood chemistry of a cross-circulating pair of dogs were made to ascertain definitely the degree of mixing that is obtained in these preparations. Typical findings of the changes in the blood chemistry of two animals which exchanged blood for 96 hours are given in the accompanying table, in which the figures represent milligrams per 100 cc.

	DOG	1 DAY BEFORE OPERATION	24 HOURS AFTER OPERATION	48 HOURS AFTER OPERATION	72 HOURS AFTER OPERATION	96 HOURS AFTER OPERATION
Chlorides	A	524	530	504	532	514
	B	564	528	502	528	522
PO_4	A	3.70	2.49	Specimen	3.40	4.28
	B	2.87	2.36	lost	3.33	4.15
Sugars	A	123	94	100	100	91
	B	208	100	100	100	93
N. P. N.	A	28.14	37.9	31.4	33.9	38.1
	B	34.7	34.5	29.6	29.7	42.3

It is seen that although before operation the respective amounts of chlorides, phosphates, etc., in the bloods of the two dogs differed moderately, after the operation these substances were found in the same concentration.

No changes in the morphology of the red blood cells or the white blood cells could be detected in the stained smears following mixing of the bloods for 48 and 96 hours. The red blood counts fell slightly from day to day in some pairs, but daily spectroscopic examinations of the plasma in eight pairs did not give any evidence of hemolysis. The fragility of the red blood cells was studied in six pairs of dogs before and after operation. No significant or constant changes could be detected. In fifteen of the experiments the cells and sera of the intended mates were matched before operation. In only one case was there prompt and extensive agglutination. This matching was repeated and the results were confirmed. Agglutination was not reciprocal. The dog whose serum agglutinated the cells of his partner died 15 minutes after the crossing of the circulations began. This animal's pulse was slow and full and his respirations were deep and regular as long as the circulations were separate. Within 2 minutes after the bloods began to mix the pulse and respirations were altered and became increasingly rapid and shallow. In another pair there was some agglutination and slight hemolysis after the matching had stood for an hour. No untoward effects were noticed, however, during the $4\frac{1}{2}$ days in which the bloods continued to mix.

The respiratory rates of cross-circulating animals are often different. Doctor Grollman, of the Department of Physiology, obtained specimens of alveolar air and determined the CO_2 tension. The results are given in the accompanying table.

PAIR	DOG	RESPIRATORY RATE PER MINUTE	ALVEOLAR CO_2 TENSION	
			Specimen	Milligrams
I	A	120	1	14.0
			2	14.4
	B	48	1	24.7
			2	23.8
II	C	52	1	28.0
			2	28.4
	D	80	1	20.4
			2	21.3

Certain other experiments were suggested by the outcome of the carotid-jugular anastomoses, which have been described above. The first of these was to suture the skins of two dogs together and to apply coaptation bands of adhesive plaster about their necks and bodies. This was done with two pairs of dogs, and in both instances the wounds broke down completely within 72 hours. The second series of correlated experiments were carotid-

carotid and jugular-jugular anastomoses. This form of operation was done three times. Twice one animal bled to death into his mate within 3 hours. In the third pair the circulatory exchange was better balanced and was maintained for $4\frac{1}{2}$ days. The third set of experiments consisted in uniting a dog's right carotid artery with its left jugular vein. This operation was done eight times, and in seven of the experiments the vessels became thrombosed within a week The arteriovenous anastomosis of the remaining dog functioned $4\frac{1}{2}$ weeks.

An early occlusion by thrombosis of end-to-end carotid-jugular anastomoses has been the experience of other workers in the field of vascular surgery. The explanation is probably to be found in the abrupt slowing of the current as the blood enters the widely dilated vein. It would be interesting to diminish the size of the vein in some way so as to produce in it a very gradual transition from the diameter of the artery to the full diameter of the vein.

SUMMARY

This paper describes the technic for crossing the circulating bloods of dogs by means of carotid-jugular anastomoses. The results of forty experiments are given. In two instances the exchange of blood was maintained for $6\frac{1}{4}$ days. The effects of cross-circulation upon the pulse rate, respiratory rate, temperature, blood pressure, blood chemistry, and red blood cells were studied. Three sets of experiments closely related to the crossed carotid-jugular anastomoses are reported.

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STUDIES ON THE ADRENAL CORTEX

I. The Effect of a Lipid Fraction upon the Life-span of Adrenalectomized Cats

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It is a well established fact that in cats and dogs, bilateral adrenal extirpation results in death within a few days. The animals exhibit a train of symptoms which has come to be recognized as typical of adrenal insufficiency. Experiments have shown, moreover, that death is due to removal of the cortical part of the suprarenal complex, and not to interference with, or loss of the medulla or adrenalin containing portion. (Biedl, 1913; Wheeler and Vincent, 1917; Houssay and Lewis, 1923; Wislocki and Crowe, 1924; Zwemer, 1927; Swingle, 1927.)

Administration of adrenalin to bilaterally operated animals has been repeatedly tried but without success, since animals so treated derive no benefit from the injections and die with characteristic symptoms of adrenal insufficiency. We have attempted numerous times to prolong the life-span of adrenalectomized cats by injections of adrenalin, employing various dosage but in no case have we noted any improvement in the condition of the animals or been able to prevent the onset of symptoms. In fact, it is our impression, although serious efforts to test the point were not made, that cats lacking adrenals are somewhat more sensitive to adrenalin than are normal animals. However this may be, it is certainly a conservative statement to say that in so far as the maintenance of life is concerned, it is the cortical and not the medullary portion of the suprarenal of mammals which is important.

Efforts to keep double operated animals alive by substitution therapy with suprarenal cortical tissue or extracts have not yielded decisive results. Very few investigators have reported success by transplantation methods (Jaffe, 1927), and but two reports have been made claiming success with extracts of fresh glands when tested upon bilaterally adrenalectomized animals.

Hartman, Brownell, Hartman, Dean and MacArthur (1928) extracted beef cortex by shaking the ground tissue with water for 15 to 20 minutes. Following isoelectric precipitation the proteins remaining in the filtrate

were salted out with sodium chloride. The salted out proteins, dissolved in water represented the extract. One cubic centimeter of the most concentrated extract was equivalent to 5 grams of fresh beef cortex. By subcutaneous semi-daily injections of the extract Hartman and co-workers stated that they were able to keep adrenalectomized cats alive for varying periods over and beyond the normal life-span of untreated adrenalectomized control animals.

Stewart and Rogoff (1929) using extract prepared according to the method of Hartman and collaborators, were unable to confirm Hartman's results. They were not able to obtain prolongation of the life-span of bilaterally adrenalectomized cats or dogs. Their data indicated that the extract-treated cats did not survive any longer than control untreated animals. On the basis of their negative findings Stewart and Rogoff concluded that Hartman's extract contained little, if any, cortical hormone.

These same investigators reported on an extract of the suprarenal glands of beef and sheep which they prepared. No data are given regarding the method of extraction, or whether or not the preparation does or does not contain adrenalin. They state that the extract when given intravenously to adrenalectomized dogs prolongs the life-span, but is ineffective when given subcutaneously or by mouth. However, examination of the data presented in their paper (1929) leads one to doubt whether their extract contained any more of the cortical hormone than that of Hartman. The average survival period of 32 adrenalectomized dogs treated daily with intravenous injections of sheep and beef adrenal cortex was 13 days (table 4, page 259). The average survival period for a series of 36 adrenalectomized control dogs not treated with extract was 9.6 days (table 2, Stewart and Rogoff, 1928).

It is our opinion that the difference between the survival periods of control and extract-treated dogs in these experiments is entirely too small to be of any significance. The quantity of active cortical hormone present in the extract must have been negligible.

So far as the writers are aware, Hartman and co-workers, and Stewart and Rogoff are the only investigators who have made any claim to have obtained an extract of the suprarenal cortex which has any effect upon the life-span of adrenalectomized animals. Critical examination of their data, however, leaves one with the conviction that the extracts contained little or no activity.

In the present confused state of our knowledge of the functional significance of the suprarenal cortex, the only reliable criterion for testing the activity of an extract is its effect upon the life-span of adrenalectomized animals. This criterion is severe and requires much time and effort, but at any rate is an excellent index of potency. If an extract fails to keep a series of double operated animals alive and in normal condition at least for

a considerable period of time over and beyond the maximum limit of the life-span of control non-treated adrenalectomized animals, we regard it as inactive. The maximum limit of the life-span of control operated cats in our colony is fifteen days, the average life-span being 7.7 days. Very rarely have we encountered accessory glands in the several hundred cats we have used in our various experiments. In our experience about 3 per cent of the animals have accessories. (Swingle, 1927.) All bilaterally adrenalectomized animals are autopsied and careful search made for accessory cortical glands.

During the past two years the writers have prepared and tested a large number of different types of cortical extracts. Varying degrees of activity were obtained from several of the extracts and one, a lipid fraction of the cortex, revealed a considerable degree of potency.

In the earlier work we employed acid agents such as acetic, hydrochloric, and sulphuric in different strengths, with and without heat; alkaline agents such as sodium hydroxide, sodium carbonate, sodium bicarbonate and ammonium hydroxide, with and without heat; acid and alkaline alcoholic extraction; desiccated cortical tissue extracted with various solvents, etc., etc. None of these various methods proved successful since but little of the active hormone was obtained. It is not improbable, however, that certain of these methods might yield potent extracts providing extensive experimentation were carried out In our experience the extracts obtained by the methods enumerated were very weak in potency compared to the lipid fraction described in this paper. This fraction is the basis of all of our further fractionation procedures and the starting point of our most active preparations. A brief note regarding the active lipid fraction has been published. (Pfiffner and Swingle, 1929; Swingle and Pfiffner, 1929.)

Each extract was tested on at least three animals simultaneously and for some of our relatively inactive extracts fifteen or twenty animals were used before the method was definitely discarded. For obvious reasons data collected in testing the many methods of extraction which were later abandoned are not included in this paper.

The method used in preparing the active lipid fraction is as follows:

The beef adrenal glands packed in ice are received in the laboratory within twenty-four hours after collection at the abattoir. After removing extraneous fat and connective tissue the glands are cut lengthwise and dissected as free as possible from medullary tissue.

The ground cortical tissue is extracted from 24 to 72 hours at room temperature with 2.5 volumes of 95 per cent ethyl alcohol. During the course of extraction the material is occasionally stirred. The extraction alcohol is removed by straining through muslin and filtering. The gland residue is pressed as dry as possible in a tincture press, ground and extracted at room temperature for 24 to 72 hours with 2 volumes (calculated from the

weight of fresh cortex) of 80 per cent ethyl alcohol. The extraction alcohol is removed in the same manner as in the first extraction. The alcoholic filtrates are concentrated individually in partial *vacuo* at an external temperature of 50–60°C. to approximately one-fifteenth of their original volumes. Each concentrate is then mixed with an equal volume of benzene and set aside in the refrigerator. The benzene solution of lipids is removed and the aqueous residues re-extracted two or three times with similar quantities of benzene. The last benzene washing is colorless or only faintly yellow. The benzene washings are combined and the benzene is removed in partial *vacuo* at an external temperature of 45–50°C. The last traces of benzene are removed by the addition of 50 to 100 cc. portions of absolute alcohol and continuing the distillation to dryness.

The lipid residue is taken up in corn oil or olive oil for injection with the aid of absolute alcohol. The alcohol is removed by distillation in partial *vacuo* at 45–50°C. Sufficient oil is incorporated so that 1 cc. represents 30 grams of fresh cortical tissue. The extract was stored in the refrigerator at 6°C. and was made up fresh each week unless otherwise indicated. It was warmed to body temperature for injection.

The lipid fraction which is being used in further purification studies is obtained by the described technique with the exception that the cortical tissue is extracted four to six days.

The cats, both control and extract-treated, were kept under the same conditions. They were housed in thermostatically heat-regulated rooms at about 78–80°F., the temperature not varying more than three degrees during the twenty-four hours. The matter of temperature control is important in any experiments involving adrenalectomized cats since such animals are very susceptible to colds and respiratory infections. The diet consisted of canned salmon fed five days a week, raw liver or kidney once each week, and milk twice a week, the latter being given on the days when salmon was fed. A few drops of cod liver oil were administered about once each week. The animals were fed but once each day and given as much as they could eat.

It has been our experience that cats kept for considerable periods in the laboratory should be treated with a vermifuge. Many of our animals were found to be infested with hook-worm. It is part of our routine procedure to give all animals coming into the laboratory 0.5 cc. of tetrachlorethylene and to repeat the dose after five days. All of our animals were operated by the same individual.

The establishment of the normal life-span of adrenalectomized control animals is the first requisite to work on cortical extracts involving length of life. It has been repeatedly pointed out by various workers that double operated animals (cats and dogs) generally remain normal for the first few days following gland extirpation. The survival period differs in different

individuals and may vary anywhere from five to fifteen days, although the majority of our animals die between the 5th and 8th day after operation.

TABLE 1

Survival period of adrenalectomized cats

SERIES	SEX	INTERVAL BETWEEN OPERATIONS	SURVIVED AFTER SECOND OPERATION
		days	*days*
E. 1	Male	23	5
E. 2	Female	23	4
E. 3	Female	25	8
E. 4	Female	25	5
E. 6	Male	24	7
F. 6	Male	31	5
F. 7	Female	26	5
F. 9	Female	28	13
F. 10	Male	26	5
F. 16	Male	13	11
F. 20	Male	12	8
H. 1	Male	10	8
H. 2	Female	10	7
H. 3	Female	10	6
H. 4	Female	10	6
H. 5	Male	8	9
H. 6	Male	7	5
H. 7	Female	7	8
H. 8	Female	8	9
H. 9	Female	8	4
H. 10	Male	7	4
H. 15	Male	12	8
H. 16	Male	32	15
H. 17	Male	28	14
H. 18	Male	22	8
H. 19	Male	36	8
H. 20	Male	7	5
H. 21	Male	12	7
H. 24	Male	10	8
H. 27	Male	8	6
H. 30	Male	9	9
H. 31	Male	9	7
H. 32	Female	7	14
H. 33	Male	7	7
H. 34	Male	7	7
H. 35	Male	8	15
H. 36	Male	7	6
H. 37	Female	15	10

The survival period of thirty-eight untreated control adrenaleotomized cats of our series ranged from 4 to 15 days with an average of 7.7 days.

These figures compare favorably with those of the best survivals reported in the literature. Table 1 shows the survival periods of our control animals. The time is given in days and is figured from the hour of operation. Fractions of days have not been recorded in the table. For instance if an animal lives five days (figured from hour of operation) and several hours beyond this time it is set down as having survived five days. If it lives five days and an additional twelve hours it is still recorded as living five days. If the animal is last seen alive at 9 p.m. and is found dead at 8 a.m. the following morning it is recorded as having died at 9 p.m. the hour when last seen alive. The exact number of hours an animal survives double adrenalectomy is of little importance—any survival period under four days is a poor case and any period over five days is a good case in so far as cats are concerned, providing the animal recovers quickly from the operation and eats and behaves normally for at least forty-eight hours before showing any symptoms.

There are two points brought out by examination of table 1, which are not without interest. The sex of the animal has no influence on the survival period, nor does the interval between first and second adrenal extirpation exert an appreciable influence on the life-span. It has been our custom to allow at least a seven day interval to elapse between removal of right and left glands.

In table 2 are shown the results of treating twenty-three double operated cats with a lipid fraction of the cortex. The survival periods vary greatly ranging as they do from 16 to 50 days. If table 1 is compared with table 2 the effect of the lipid extract upon the life-span is clearly brought out. None of our control cats survived over fifteen days, and none of the extract-treated animals survived less than sixteen days. The average life-span for controls was 7.7 days (38 animals) the average for the extract treated was 27.8 days (23 animals).

The extract-treated animals were normal, they ate, played about and remained active up until a few days before death (protocol 1). The onset of adrenal insufficiency symptoms was in general quite abrupt and when symptoms appeared we were unable by increasing the dosage to bring the animals back to normal. Death invariably occurred within two or three days from the date of first refusal of food. All extract-treated cats received two injections daily, one-half the daily kilogram dose being administered in the morning, the other half in the evening, except G 31, G 32, G 33 and G 34 of table 2. These animals received the entire daily dose in one injection.

The injections were begun twenty-four hours after the second operation in all except two cases (G 31 and G 32, table 2), when the first injection was given at the end of the fourth day following removal of the second gland. All of the animals later died of adrenal insufficiency aggravated by abscess formation at the site of the injections.

It is interesting to note that after the potent fraction is prepared and placed in oil it retains its potency for at least three weeks if kept at 6°C. Several cats were used to test the keeping quality of the extract in oil.

TABLE 2

Survival period of adrenalectomized cats treated with subcutaneous injections of a lipid extract

SERIES	SEX	INTERVAL BETWEEN OPERATIONS	SURVIVED AFTER SECOND OPERATION	REMARKS
		days	*days*	
G. 11	Male	8	24	1 cc. extract per kilo daily. Abscess formed
G. 12	Male	20	29	1 cc. extract per kilo daily. Abscess formed
G. 13	Male	8	20	1 cc. per kilo daily. Abscess formed
G. 14	Male	8	17	1 cc. per kilo daily. Abscess formed
G. 16	Male	8	20	1 cc. per kilo daily. Abscess formed
G. 17	Female	8	16	1 cc. per kilo daily. Abscess formed
G. 18	Female	8	26	1 cc. per kilo daily. Abscess formed
G. 19	Female	8	28	1 cc. per kilo daily. Abscess formed
G. 20	Male	8	32	1 cc. per kilo daily. Abscess formed
G. 21	Female	8	39	1 cc. per kilo daily
G. 22	Male	7	44	1 cc. per kilo daily. Extract 3 weeks old at end of 44th day
G. 23	Male	7	50	1 cc. per kilo daily. Extract 3 weeks old at death of animal. Abscess formed
G. 24	Male	7	18	1 cc. per kilo by mouth daily
G. 25	Male	19	18	1 cc. per kilo daily
G. 26	Female	15	42	0.5 cc. per kilo daily. Died pneumonia
G. 27	Male	7	44	0.5 cc. per kilo daily
G. 28	Female	12	21	0.5 cc. per kilo daily
G. 29	Female	10	29	1 cc. per kilo. Cat sacrificed. Excellent condition to death. See protocol in text
G. 30	Male	10	28	1 cc. per kilo. Cat killed by adrenalin injections
G. 31	Male	26	19	0.5 per cat daily. 1st injection 4 days after 2nd operation. Pneumonia
G. 32	Male	26	20	0.5 cc. per cat daily. 1st injection 4 days after 2nd operation. Pneumonia
G. 33	Female	28	23	1 cc. per cat daily
G. 34	Female	12	33	0.5 cc. per kilo daily

The usual extract was prepared and injected daily, the same sample of extract being used until the animals died. One animal survived 19 days (G 31, table 2) and received but 0.5 cc. of extract per day. The extract was not injected until the fourth day following the second operation when the cat first refused food. Another animal operated the same day survived

20 days and received the same dose. It also first received the extract on the fourth day after operation (G 32, table 2). A third animal operated the same day as the previous two cats, survived 23 days on the same batch of extract but received 1 cc. daily (G 33, table 2) and received the first injection twenty-four hours after operation.

The keeping quality of the hormone in ethyl alcohol was tested on two animals. The cats were started on a sample of extract. After some days' treatment the extract was used up and another lot made up from cortex tissue which had been standing in alcohol at room temperature for two weeks. This lot of extract kept the animals in excellent condition for a little over three weeks. The animals died within two days of one another. The survival period of the two animals was 42 days (G 26) and 44 days (G 27). These cases offer strong evidence that the active principle of the cortex remains stable in alcohol for at least two weeks at room temperature and when prepared in extract form in oil retains its potency for approximately three weeks.

In all of our cases complications arose following repeated injections of the extract, due to formation of abscesses at the site of injection. Animals which survived twenty-five to thirty days generally had several such abscesses, some open sores, some large lumpy masses. When large areas of the subcutaneous tissues became involved the cats developed symptoms of adrenal insufficiency and died. Autopsy revealed that little if any of the later injected extract had been absorbed. Most of it could be recovered at the site of injection. Consequently we have found it extremely difficult to keep animals alive for periods longer than 45 days with this crude extract.

Adrenalectomized animals are sensitive to any type of infection such as colds, or abscess formation such as just described. In later work we used olive oil instead of corn oil as a medium for injecting the extract. As a result of changing oils, abscess formation was materially reduced and the cats remained in better condition.

The presence of appreciable quantities of adrenalin in cortical tissue has proved to be a source of serious difficulty, especially so since adrenalectomized animals appear to be somewhat hypersensitive to adrenalin. It was thought that this difficulty might be surmounted by dissection of the glands at the slaughterhouse immediately after removal from the animals. The data presented in table 3 show quite clearly that no advantage would be gained by such a procedure, since cortical tissue dissected at the slaughterhouse contains almost as much adrenalin as cortical tissue obtained from glands which were dissected 20 hours after removal from the animal. It should be noted that the glands used in the above analyses were handled very carefully so as to keep diffusion processes at a minimum. In routine work in the laboratory it is impractical to dissect very carefully large numbers of adrenal glands. Consequently the tissue used in the prepara-

tion of our extracts contains more adrenalin than the data in table 3 would indicate. The ground cortical tissue in routine practice assays about 2 to 2.5 mgm. of adrenalin per gram of tissue using the colorimetric method of Folin, Cannon, and Denis (1913). Approximately 30 to 40 per cent of the adrenalin present is extracted by the alcoholic treatment. As a result the aqueous concentrates after the removal of alcohol are highly toxic. Because of its toxic properties we were unable to demonstrate the presence of the cortical hormone in this material.

About 3 to 5 per cent of the adrenalin present in the cortical tissue is found in the benzene soluble fraction. Adrenalin is insoluble in benzene. However, it is soluble to a certain extent in a solution of lipid materials in that solvent. The chief disturbing factor in this connection appears to be phospholipid. Neutral fat, free fatty acids and cholesterol have a slight influence. This change in solvent properties probably accounts for the

TABLE 3

Adrenalin content of adrenal glands (beef)

		MILLIGRAM ADRENALIN PER GRAM TISSUE (BIO-ASSAY)
Cortex dissected from medulla while glands still warm (about 20 minutes after death of animal)	Cortex	0.7
	Medulla	10.0
Whole glands stored in refrigerator (8°C.) for 20 hours before dissection	Cortex	1.0
	Medulla	12.0

presence of adrenalin in the benzene soluble fraction. On the other hand, it is possible that a fraction of the adrenalin may exist in the tissue in a form which is soluble in benzene. Similar solubility behaviour of adrenalin was encountered with the many other solvents examined including ether, petroleum ether, chloroform, ethyl acetate, acetone, hexane, butyl alcohol, carbon disulfide, and carbon tetrachloride.

One cubic centimeter of the crude extract used in this study contained on an average 2 mgm. of adrenalin. This quantity of extract is equivalent to 30 grams of cortical tissue which contained on an average 60 mgm. of adrenalin. In the procedure used therefore from 96 to 97 per cent of the adrenalin is removed.

The quantity of adrenalin present in the crude extract is capable of inducing physiological changes in the organism such as elevation of blood pressure, rise in blood sugar, etc. However, the extract is given subcutaneously in oil and is absorbed rather slowly over a considerable period. We never give more than 1 cc. per kilo per day per animal and have not noted any changes in the behaviour of the animals following

injection. However, if large doses are given, doses of 5 to 6 cc., the animals display typical symptoms of adrenalin overdosage. They become sluggish and lose their appetites.

It should be emphasized that our primary object in this study was the conclusive demonstration of the presence of the cortical hormone in the crude lipid fraction. No attempt was made to determine the minimum dosage or the minimum frequency of injection necessary to maintain life.

SUMMARY AND CONCLUSIONS

By use of an alcoholic-benzene extraction process a crude lipid fraction of the suprarenal cortex of beef has been obtained, which when injected subcutaneously will prolong considerably the survival period of bilaterally adrenalectomized cats.

The cortical hormone is stable in ethyl alcohol for a period of at least two weeks, and the crude lipid fraction when prepared in oil retains its potency for about three weeks when kept at 6°C.

The active lipid fraction is not adrenalin-free.

In so far as the writers are aware this is the first conclusive demonstration that the hormone of the suprarenal cortex which is essential for the maintenance of life can be extracted with lipid solvents.

Protocol G 29, female. 2-1-'29, r. adrenal removed. 2-10-'29, l. adrenal removed. Quick recovery from ether. Given 2 cc. daily subcutaneously of lipid extract suprarenal cortex in two equal doses. 2-10 to 2-12, cat perfectly normal, eats heartily, plays about, and is very active. 2-18 to 3-3, animal in such excellent condition that presence of accessory gland suspected. 3-3, extract discontinued, 3-6, animal first refused food, and showed first symptoms of adrenal insufficiency. 3-10, animal prostrate and verging on coma at 9 a.m. Died 4 p.m. Autopsy revealed no traces of accessory adrenal tissue. Total survival period 29 days.

Protocol. Cat G 22, male. 7-8-'29. Weight 2540 grams. R. adrenal removed. 7-15-'29. Weight 2535 grams. L. adrenal removed. 7-16-'29. Ate well. One and one-fourth cubic centimeter extract given subcutaneously twice daily unless otherwise noted. 7-27-'29 refused food, cat "droopy." 7-28-'29. Appetite returned, ate heartily, appears normal, plays about in lively fashion. 8-12-'29 extract discontinued for three days. Cat normal. 8-15-'29 injections resumed since cat refused food. Abscesses formed at site of injection. 8-25-'29 injections discontinued for two days. Cat normal. Injections resumed on 27th. 8-27-'29, cat shows typical adrenal symptoms—weakness, loss of appetite, lethargy. Several large abscesses at site of previous injections present. 8-28-'29 animal presented marked symptoms adrenal insufficiency. Bled (cardiac puncture) for urea and sugar, 8 a.m.; cat died 12 m., weight 2055 grams. Autopsy revealed no accessory tissue. Blood sugar four hours previous to death, 45 mgm. per cent; urea 24 mgm. per cent. No sugar in urine at time of death. Lived 44 days after second adrenal removed. Diagnosis—adrenal insufficiency aggravated by abscess formation.

This work was begun at the Zoölogical Laboratory of the State University of Iowa, continued on through the summer of 1929 at the Biological

Laboratory at Cold Spring Harbor, Long Island, and is now in progress at the Biological Laboratory of Princeton University. We take pleasure in acknowledging our indebtedness to Dr. Oliver Kamm, of Parke, Davis & Company, for his very generous coöperation.

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STUDIES ON THE ADRENAL CORTEX

II. An Aqueous Extract of the Adrenal Cortex Which Maintains the Life of Bilaterally Adrenalectomized Cats

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It was pointed out in a previous communication (paper I of this series, This Journal) that in extracting adrenal cortex, the cortical hormone followed the lipid fraction, and our earlier work was concerned solely with testing the activity of this lipid extract. It soon became evident that the lipid fraction, taken up in olive oil and administered subcutaneously, was unsuitable for repeated injections. The material although quite potent when tested upon the prolongation of the life-span of adrenalectomized cats, caused severe local irritation at the site of injection. The animals quite frequently developed abscesses, or else hard lumps formed, which occasionally broke down, causing sloughing of the skin. We found it difficult to keep adrenalectomized cats in good condition, much beyond thirty-five to forty days when treated with the extract, owing to abscess formation and difficulties of administration. Because of these facts further experiments with the crude lipid fraction, as such, were abandoned and fractionation studies undertaken with the view of eventually obtaining a potent extract in aqueous form suitable for subcutaneous or intravenous use on animals or man.

A complete account of the earlier work is given in paper I of this series, together with details of the criterion of activity employed for testing the extracts, the normal survival period of adrenalectomized animals, methods of operation, diet of the animals, etc. In so far as the physiological testing of the extracts is concerned, exactly the same procedure was followed as outlined in paper I of this series, and the reader is referred to the previous communication for details. Brief preliminary accounts of various aspects of this work have been published (Science, 1930, lxxi, 321; lxxi, 489; lxxii, 75). Early in the work we elected to obtain the crude lipid fraction with an alcohol-benzene extraction process since it was found that the cortical hormone was stable in either of these solvents. In further purification studies, various methods of fractionation were investigated. The method of fractionation reported here proved to be the most satisfactory from the

standpoint of consistent yield and purity. The lipid fraction obtained by the alcohol-benzene extraction process is treated with acetone. The acetone insoluble material consisting chiefly of phospholipids is inert. The acetone soluble fraction is further purified by selective distribution between seventy per cent alcohol and petroleum-ether. The petroleum-ether soluble fraction is inactive. The seventy per cent alcohol soluble fraction contains the cortical hormone along with some adrenalin and inert material, including appreciable amounts of pigment.

The method of preparation can best be illustrated by citing the following typical experiment:

Ten pounds of beef adrenal glands packed in ice were received in the laboratory. They had been collected at the slaughter house the previous day. After removing the fat and connective tissue, the glands were split lengthwise and the medullary tissue scraped out. The cortical tissue weighed 3000 grams. It was passed through an ordinary meat grinder using the finest cutter. The ground cortical tissue was mixed with 2.5 volumes of 95 per cent alcohol and allowed to macerate at room temperature with occasional stirring for three days. The mixture was strained through muslin and the tissue residue was thoroughly expressed in a tincture press. The strained fluid was filtered through soft paper. The gland residue was reground and extracted in a similar manner for three days with 2 volumes (based on fresh gland weight) of 80 per cent alcohol. The alcoholic extracts were concentrated separately in partial *vacuo* at an external temperature of 50–60°C. to about one-fifteenth their original volumes. Each aqueous residue was transferred to a cylinder with an equal quantity of benzene. After thorough mixing, the material was set aside in the refrigerator to settle. From this stage on, all material was kept in the refrigerator except when under manipulation. The benzene solution was removed and the washing repeated until the last benzene washing was colorless or only slightly yellow. Usually two or three washings are sufficient. The benzene washings were all combined and the benzene was completely removed in partial *vacuo* at an external temperature of 45–50°C. The brown semi-solid lipid residue weighed 81.0 grams.

To the residue in the flask were added 500 cc. of acetone. The material was thoroughly rubbed and set aside in the refrigerator for 24 hours with occasional rubbing. The acetone solution was decanted and the residue re-extracted in the same manner. After decanting the second acetone extractives, the residue was transferred to a mortar and rubbed with five 100 cc. portions of fresh cold acetone. The acetone was removed in partial *vacuo* at an external temperature of 45–50°C. This fraction weighed 14.7 grams.

The residue was transferred to a separatory funnel with 30 cc. of petroleum-ether (B.P. 30 or 40–60°) and 74 cc. of 95 per cent alcohol. Twenty-

six cubic centimeters of distilled water were added and the contents of the funnel gently mixed. The 70 per cent alcohol layer was washed five times with 30 cc. portions of petroleum ether. One cubic centimeter of the fifth washing gave a negative Lieberman-Burchard color reaction for cholesterol. The petroleum-ether solution and washings were returned to the original flask and the petroleum-ether removed in partial *vacuo* at an external temperature of 40–50°C. The distribution procedure was repeated. The petroleum-ether solution resulting from the second distribution was run into another separatory funnel, 74 cc. of 95 per cent alcohol were added and then 26 cc. of distilled water. The petroleum-ether solution resulting from the third distribution was treated similarly. This gave a total of four distributions. The alcoholic solutions were washed successively five times with 30 cc. portions of petroleum-ether in the order 2nd, 3rd, and 4th distributions. The alcoholic solutions of the 3rd and 4th distributions were then washed twice with fresh 30 cc. portions of petroleum-ether. Rather stable emulsions may be encountered if the technique of distributing and washing is not correctly executed. The inactive petroleum-ether soluble material weighed 13.2 grams. The 70 per cent alcohol soluble material weighed 1.489 grams.

The alcoholic solutions were combined, the alcohol removed in partial *vacuo* at an external temperature of 45–50°C. Distillation was continued to a volume of about 65 cc. Distilled water was added to make the volume 100 cc. One cubic centimeter of this extract represented 30 grams of fresh cortex. The extract was allowed to stand in the refrigerator over night, centrifuged, decanted, and filtered through a Seitz filter. The clear reddish-brown extract contained 0.5809 gram of solids. The pH (quinhydrone) was 5.2. Each cubic centimeter assayed 0.38 mgm. of adrenalin colorimetrically (Folin, Cannon and Denis, 1913) and 0.24 mgm. of adrenalin biologically (blood pressure-dog).

The three kilograms of freshly dissected cortex contained, according to colorimetric assay, 6.9 grams of adrenalin. The finished extract contained 24 milligrams. Approximately 99.6 per cent of the adrenalin was removed by this method of fractionation. In a series of 12 preparations (1 cc. equivalent to 30 grams of fresh cortex), the average colorimetric adrenalin assay was 0.36 mgm. per cc., the minimum 0.19 mgm., the maximum 0.50 mgm. The average adrenalin content measured by bio-assay (blood pressure-dog) was 0.28 mgm. per cc., the minimum 0.17 mgm., the maximum 0.37 mgm. The difference between the assays by the two methods is due to the presence in the extract of alkaline phosphotungstate reducing substances other than adrenalin. The solid content of the extracts varied between 4.4 and 8.1 mgm. per cc., with an average of 5.9 mgm. The pH (quinhydrone) varied between 4.60 and 5.75 with an average of 5.15. An extract considerably lower in adrenalin and solid content

can be obtained by modifying the technique so as to make a single alcohol extraction of the tissue, a single acetone extraction of the crude lipid fraction and only one distribution. However, our experience has been that the yield of cortical hormone is considerably less. Data on these and other points of extraction and further purification will be presented in a separate communication. The aqueous extracts used in this study were prepared by the technique outlined above.

The steps in the fractionation procedure were checked by testing the several fractions on the survival period of adrenalectomized cats. In testing the activity of the acetone soluble, acetone insoluble, and petroleum-ether soluble fractions, it was necessary to administer them dissolved in olive oil. Solution was effected with the aid of absolute alcohol which was subsequently removed by distillation in partial *vacuo* at 45°C. All extracts were prepared fresh each week, unless otherwise specified, and administered subcutaneously.

In order to make the tests on the several fractions comparable, all the extracts were prepared so that 1 cc. represented the amount of respective fraction obtained from 30 grams of fresh cortical tissue. The water insoluble fraction removed by Seitz filtration, was not tested separately. This point was checked by treating adrenalectomized animals for a few weeks with filtered extract and then for a few weeks with unfiltered extract. Suffice it to say here that no noticeable difference in the well-being of the animals was observed. It is probable that some cortical hormone is removed by Seitz filtration, but little can be said on this point since the only available criterion of activity has more of a qualitative than a quantitative significance.

It is quite obvious that the cortical hormone lends itself to fractionation methods similar in a general way, to those employed by Ralls, Jordan and Doisy (1926) for the ovarian hormone, Gallagher and Koch (1930) for the testicular hormone, and Allen (1930) for progestin.

About fifty lots of extract have been prepared, using the previously described technique. As far as we could judge from the behaviour of the test animals, all the extracts were about equally potent. In several instances the cortical tissue had been stored in alcohol at room temperature for two or three weeks and the crude benzene-soluble fraction in benzene from four to six weeks, at 8°C. The acetone-soluble fraction was routinely stored in acetone for two or three days at 8°C. Each lot of glands was in process at least 12 to 14 days. The cortical hormone is apparently quite stable under the conditions outlined.

The results obtained by treating adrenalectomized cats with one (cats A1, A2 and A3) and two (cats A4 and A5) daily injections of the acetone soluble fraction are given in table 1. They show quite clearly that this fraction is highly active. Animals so treated do not develop symptoms

and remain in normal health. This fraction is unsatisfactory for repeated use however, since repeated injections sooner or later give rise to hard lumps under the skin at the site of injection. Occasionally these lumps may break down and the skin slough off, exposing a soft cheese-like mass

TABLE 1

Survival period of adrenalectomized cats treated with the acetone soluble fraction

SERIES	SEX	INTERVAL BETWEEN OPERATION	SURVIVAL AFTER 2ND OPERATION	REMARKS
		days	*days*	
A-1	Female	28	33	1.5 cc. once daily. Weight 3000 grams. Extract discontinued on 33rd day. Animal later died adrenal insufficiency
A-2	Male	8	50	1.5 cc. once daily. Weight 3185 grams. Extract discontinued on 50th day. Cat later died adrenal insufficiency
A-3	Female	9	30	1.5 cc. once daily. Weight 3420 grams. Extract discontinued on 30th day. Cat later died of adrenal insufficiency
A-4	Female	8	30	1.5 cc. twice daily. Weight 3330 grams. Extract discontinued on 30th day. Died later of adrenal insufficiency
A-5	Male	8	30	2 cc. twice daily. Extract discontinued on 30th day. Death from adrenal insufficiency later

TABLE 2

Survival period of adrenalectomized cats treated with the acetone insoluble fraction

SERIES	SEX	INTERVAL BETWEEN OPERATIONS	SURVIVAL AFTER 2ND OPERATION	REMARKS
		days	*days*	
I-1	Female	8	5	1.5 cc. twice daily. Weight 3015 grams. Typical symptoms
I-2	Female	7	9	1.5 cc. twice daily. Weight 2000 grams. Typical symptoms
I-3	Female	7	3	1.5 cc. twice daily. Weight 2100 grams. Typical symptoms. Animal pregnant

underneath. None of our animals developed any symptoms of adrenal insufficiency while on this fraction, but we made no effort to keep them indefinitely. At the end of thirty days, the treatment was discontinued except in one case (A2). This cat was treated for 50 days. All animals gained weight while receiving the extract.

The conclusive results obtained from this series of five cats demonstrated to our satisfaction at any rate, that the active principle of the cortex is acetone-soluble. We did not feel justified in continuing the experiment further.

Although it was evident that the acetone-soluble fraction contained the hormone, it still remained necessary to test the acetone-insoluble fraction for activity since some of the active principle may have remained in the insoluble fraction. The results of testing this fraction are shown in table 2.

Three animals were used in the experiment (table 2) and each received 1.5 cc. of the material subcutaneously twice daily. All three cats died of adrenal insufficiency within a short time. It seems evident that this fraction does not contain the active principle of the cortex. Compare tables 1 and 2.

TABLE 3

Survival period of adrenalectomized cats treated with the petroleum-ether soluble fraction

SERIES	SEX	INTERVAL BETWEEN OPERATIONS	SURVIVAL AFTER 2ND OPERATION	REMARKS
		days	*days*	
P-1	Male	10	22	1.5 cc. twice daily. Weight 2725 grams. Typical symptoms
P-2	Female	43	19	1.5 cc. twice daily. Weight 2360 grams. Typical symptoms
P-3	Female	8	8	1.5 cc. twice daily. Weight 2450 grams. Typical symptoms
P-4	Male	8	7	1.5 cc. twice daily. Weight 2155 grams. Typical symptoms
P-5	Male	10	4	1.5 cc. twice daily. Weight 1970 grams. Typical symptoms

Cats P1 and P2 of table 3 were treated with the petroleum-ether soluble fraction obtained following a single distribution of the acetone-soluble fraction between petroleum-ether and 70 per cent alcohol. Cats P3, P4, and P5 were treated with a similar fraction obtained after four distributions. The animals receiving the fraction following a single distribution survived much longer than those on the same fraction after four distributions. It is evident that one distribution does not completely remove all of the cortical hormone, whereas repeated distributions do remove it. The evidence seems clear that the petroleum-ether soluble fraction is inactive after four distributions, the hormone passing into the alcohol soluble fraction.

At the same time that the tests with the petroleum-ether soluble fraction were in progress, similar experiments were under way, in which the alcohol-

TABLE 4

Survival period of adrenalectomized cats treated with the alcohol-soluble fraction

SERIES	SEX	INTERVAL BETWEEN OPERATIONS	SURVIVAL AFTER 2ND OPERATION	REMARKS
		days	*days*	
M-1	Female	7	50	1.5 cc. twice daily. Weight 2555 grams. Animal sacrificed on 50th day for autopsy. Accessory tissue not found
M-2	Female	6	50	1 cc. twice daily. Weight 1675 grams. Sacrificed on 50th day. No accessories present
M-3	Male	8	50	1.5 cc. twice daily. Weight 2725 grams. Sacrificed on 50th day. No accessories present
M-4	Male	8	47	1 cc. twice daily. Weight 2290 grams. Died pneumonia. No symptoms adrenal insufficiency noted
M-5	Male	8	109	1.5 cc. twice daily. Weight 3185 grams. Extract discontinued on 100th day
M-6	Female	12	113	1.5 cc. twice daily. Weight 2270 grams. Extract discontinued on 100th day
M-7	Male	18	112	1.5 cc. twice daily. Weight 2810 grams. Treatment discontinued on 100th day
M-8	Female	6	163	1.5 cc. twice daily. Weight 2700 grams. Extract discontinued on 100th day
M-9	Male	7	107	1.5 cc. twice daily. Weight 3200 grams, when treatment began. Extract discontinued on 100th day
M-10	Male	7	115	1.5 cc. twice daily. Weight 2835 grams. Extract discontinued on 100th day
M-11	Male	6	125	1.5 cc. twice daily. Weight 2905 grams. Extract discontinued on 100th day
M-12	Male	7	56	1 cc. twice daily. Weight 2180 grams. Cause of death unknown. Never presented symptoms. Maximum weight at death. Died in fit. No accessory tissue present
M-13	Male	10	111	1.5 cc. twice daily. Weight 2720 grams. Extract discontinued on 100th day
M-14	Male	8	118	1.5 cc. twice daily. Weight 3102 grams. Extract discontinued on 100th day
M-15	Male	10	58	1.5 cc. twice daily. Weight 3330 grams. Developed severe generalized infection. Killed. No accessory glands present
M-16	Male	10	117	1.5 cc. twice daily. Weight 2630 grams. Extract discontinued on 100th day
M-17	Female	9	56	1.5 cc. twice daily. Weight 3420 grams. Developed severe infection. Killed. No accessory tissue present
M-18	Male	10	117	1.5 cc. twice daily. Weight 3730 grams. Extract discontinued on 100th day
M-19	Male	8	120	1.5 cc. twice daily. Weight 3215 grams. Extract discontinued on 100th day

soluble fraction was employed. The results obtained with the latter were so consistent and striking that further testing of other types of extract were discontinued. The data obtained from tests of the alcohol-soluble fraction are given in table 4.

The series of 19 cats listed in table 4 shows in a striking manner that the alcohol-soluble fraction contains the hormone of the suprarenal cortex. All of the earlier fractions tested were administered in olive oil, except those described in paper I of this series. Considerable difficulty was experienced with abscess formation. With use of the aqueous preparation of the alcohol-soluble fraction, most of this trouble was eliminated. Our only precautions in injecting the material were to use a clean syringe and needles, washed with alcohol. Occasionally infections would form at the site of injection, but these could be readily cared for by irrigation with strong chlorazene solution in warm water. We made no effort to sterilize the material for injection. Despite the absence of aseptic technique, we lost but two animals in this series from infections (M 15 and M 17—table 4).

All of the animals in this series, except M2 and M4 received the same daily dose, viz., 1.5 cc. twice daily, administered at 9 a.m. and at 5 p.m. The results obtained by this treatment on adrenalectomized cats were so striking and consistent that three animals were sacrificed on the 50th day of survival in order to satisfy ourselves that no question of accessory cortical glands was involved. (See table 4—animals M-1, M-2 and M-3.) No trace of accessory tissue was found, so that the remainder of the series which were on hand at the time were continued on the treatment. Since all survived in excellent condition and gained weight rapidly, it became obvious that they would probably survive indefinitely so long as the treatment continued. As our laboratory facilities for keeping animals were extremely limited, it became necessary to fix an arbitrary period of survival at the end of which the extract treatment would be discontinued, and the animals permitted to die. A survival period of 100 days was selected as the test period, and the administration of extract stopped at the end of this interval.

A summary of the results is given in table 4. It should be borne in mind, however, in evaluating the data in this table, that in all cases extract treatment was discontinued on the 100th day of survival following removal of the second adrenal gland. In all of our cases, with the exception of animals M-15 and M-17 which died from generalized infections caused by repeated injections of non-sterile material, and M-12 which died suddenly in convulsions, the animals were in perfect health at the time of cessation of extract treatment. They gained steadily in weight except during those intervals when colds developed and such slumps as occurred as a result of respiratory infections were more than made up for when the animals shook off the cold.

The behavior of such long surviving cats was perfectly normal so far as we were able to judge. They ate heartily, played about, fought with one another, and in two instances, males were observed to attempt copulation with females newly introduced into the laboratory. Persons viewing the animals, and without knowledge of their history were unable to distinguish the adrenalectomized extract treated cats from normal unoperated animals when the site of operation was covered in both types of animals. The only difference we have noted between the long surviving animals and untreated bilaterally adrenalectomized or normal cats is a tendency to more frequent urination on the part of the extract treated animals. Whether this has any significance or not, we are unable to state at the present time.

After withdrawal of the extract, the animals generally remain normal for a varying period. Some develop symptoms and die with typical symptoms of adrenal insufficiency within the first two weeks (M5, M6, M7, M9, M13, table 4). Others remain active for longer periods (M8, M10, M11, M14, M16, M18, M19). With one exception (M8) all the cats died from adrenal insufficiency within 25 days following cessation of extract treatment. Five died within two weeks, six within three weeks, one survived 25 days (M11) and one 63 days (M8).

The animal surviving 63 days is an interesting case, and is exceptional in our series. It is noteworthy that almost immediately after discontinuing the extract treatment, the weight fell off rapidly. Because of this striking weight loss we decided against killing the cat and searching for an accessory cortical gland. The animal died with characteristic symptoms of adrenal insufficiency on the 63rd day after withdrawal of extract, losing 1076 grams in weight during this interval, and at no time presenting any symptoms of cold or respiratory or other infection. The appetite remained excellent up to within five days of death. Careful autopsy failed to reveal any traces of accessory cortical tissue.

It is interesting in this connection, to compare the protocol of cat M-8 with that of M-X, an animal which survived for 47 days following withdrawal of extract. At the end of this interval the animal was in such excellent condition that it was killed for autopsy. An accessory cortical gland was found attached to the left renal vein. This animal continued to gain weight steadily after discontinuing the extract, in contrast to animal M-8 which lost over a thousand grams' weight within 63 days.

During the past seven years, one of the present writers has operated and studied over five hundred adrenalectomized cats, and it is our experience that not over three or four per cent of cats have accessory cortical glands. Many so-called accessory glands represent merely pieces of the original gland, inadvertently left behind at the time of operation.

The autopsy findings on the long surviving cats permitted to die of adrenal

insufficiency following cessation of extract treatment, do not differ from those reported by several authors for animals surviving but a week or ten days after gland extirpation. This is to be expected, since our animals were normal in every respect, so long as they received extract, and were not in any sense suffering from a chronic condition of adrenal insufficiency. The protocols of the M series of cats summarize briefly some of the principal facts brought out by post-mortem examination. We would, however, like to call attention to the greatly enlarged thymus gland of our long surviving animals. In most cases, the thymus glands were extremely large and associated with the enlarged thymus was a marked enlargement of the mesenteric lymph nodes.

The liver and spleen were usually greatly congested and very dark in color. The pancreas was in some cases slightly congested and in others markedly so. There was some variation among the animals in this respect. Bile was usually present in the intestinal tract, sometimes in considerable quantities. In no case was blood found in the lumen of the gut, although in some cases the walls of the small intestine were congested. The kidney, thyroid and parathyroids appeared normal upon gross examination.

Striking weight gains were exhibited by all of the extract treated animals and a remarkable weight loss occurred in our cases following withdrawal of extract. Weight loss is a well known symptom of adrenal insufficiency. The animals will lose hundreds of grams in weight within a few days and yet continue to eat every day. The weight of the cats declines in a remarkable manner and is not due to the fact that the animals decline food, for in many cases adrenalectomized cats will continue to eat their usual amount of food each day, up to within two or three days of death. We have observed several animals which ate heartily and died of typical symptoms of adrenal insufficiency within twelve hours. There appears to be a loss of water from the animals and it seems probable that the dehydration may account for a considerable fraction of the weight loss.

So far no mention has been made regarding maximum and minimum dosage. With the type of extract employed in the present set of experiments, it was not possible to obtain data relative to maximum dosage or over-dosage. The material contained appreciable quantities of adrenalin, as was previously mentioned, and if more than 4 cc. were given at a single injection, symptoms of adrenalin over-dosage appeared. Our animals received 1.5 cc. at a time and this dose was given but twice daily. They did not at any time present adrenalin symptoms. We have recently completed a set of experiments employing a modification of the type of extract used in the present work and have found that very large doses of the material can be injected intraperitoneally and intravenously without harmful effect. The modified technique removes all but a trace of adrenalin.

Owing to the pressure of other work we have not found sufficient time to

devote to a study of minimum dosage of the extract. However, we have one very suggestive case which is of interest. The following abbreviated protocol gives the essential points.

Protocol M-9. Adult male. Weight 3815 grams. R. gland removed March 4. L. adrenal removed March 11. Weight 3710 grams. Animal not injected for 7 days during which time it developed severe symptoms of adrenal insufficiency such as anorexia, weakness, unsteadiness in legs when walking, so that it swayed from side to side. Weight dropped to 3200 grams by March 18. On this date animal was injected three times daily with a modification of the type of extract used in the experiments described in this paper, the adrenalin content having been reduced to less than 0.01 mgm. per cc. of extract. The symptoms promptly disappeared and the animal returned to normal condition and weight. It was then taken off the modified extract and injected twice daily with 1.5 cc. of the regular extract employed on the other cats. April 28, cat normal, weight 3885 grams. June 10, cat normal, weight 3970 grams. Dosage reduced to 1 cc. given once daily. The animal's appetite remained normal but its weight began to drop sharply within a few days. June 19, animal had reached its 100th day of survival. Extract discontinued. Weight 3640 grams. From this time on cat declined rapidly and death from adrenal insufficiency resulted 7 days after discontinuing the extract. During the 7 day interval from the time of reducing the dosage to approximately 0.25 cc. per kilogram once daily, to date of entirely discontinuing treatment, the cat lost 330 grams. The animal was entirely free from cold or other infection. The animal survived cessation of extract treatment but 7 days—the shortest period encountered in our series.

The evidence presented by M-9 indicates that 0.25 cc. per kilogram of body weight given but once daily, in a single dose, is inadequate to maintain the animal in perfect condition. It is our opinion that this quantity of extract represents approximately the minimum dose that can be given. No doubt there is considerable variation as to dosage requirements among cats, but about 0.5 cc. per kilo represents the amount of extract required to maintain normal health.

The type of extract discussed in this paper has been tested in two severe cases of Addison's disease, by Dr. L. G. Rowntree of the Mayo Clinic (Rowntree, Kintner, and Lymburner, 1930).

SUMMARY AND CONCLUSIONS

Fractionation studies of a crude lipid extract obtained by alcoholic-benzene extraction of the suprarenal glands of beef were undertaken. The criterion of activity employed was the effect upon the life-span of bilaterally adrenalectomized cats. The following facts were obtained:

a. The acetone soluble fraction contains the cortical hormone and is highly active, but offers serious difficulties in administration.

b. The acetone insoluble fraction is inactive since it is entirely without beneficial effect upon the life span of adrenalectomized animals.

c. The petroleum-ether-soluble fraction following a single distribution of the acetone-soluble fraction between petroleum-ether and 70 per cent

alcohol contains a small amount of the hormone. After four such distributions the petroleum-ether fraction is without activity.

d. The alcohol soluble fraction obtained after distribution between petroleum-ether and 70 per cent alcohol contains the hormone of the suprarenal cortex. This fraction will maintain adrenalectomized cats indefinitely in normal health, so long as the extract is administered. Following cessation of treatment (an arbitrarily selected period of 100 days was used) such animals die within a short period with typical symptoms of adrenal insufficiency.

Potent extracts have been prepared from adrenal cortex stored in alcohol at room temperature for periods of two to three weeks. No deterioration in activity was observed in active fractions stored in alcohol or benzene at 8°C. from four to six weeks. Active fractions can be safely stored in acetone for several days. Our tests indicate that there is no loss of activity in aqueous extracts in seven to fourteen days when stored at 8°C. without a preservative. We have preferred to use fresh extracts each week. Observations on keeping qualities of aqueous extracts with and without preservatives will be reported later.

The aqueous extract is not adrenalin free. The scheme of fractionation eliminates on an average about 99.6 per cent of the adrenalin present in the fresh cortical tissue. The extract is protein free.

The writers prefer to use the term *cortical hormone* pending definite knowledge of the chemical nature or physiological function of the hormone or hormones involved.

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PROTOCOLS OF CATS TREATED WITH THE ALCOHOL-SOLUBLE FRACTION. *Series M.*

M 1. Adult female. Weight 2820 grams. December 6, right adrenal removed. December 13, left adrenal removed. Weight 2555. December 14, extract treatment begun; 1.5 cc. given subcutaneously at 9 a.m. and 5 p.m. January 19, animal normal. Weight 2755 grams. February 1, animal in perfect condition. Weight 2900 grams. Animal in such perfect condition it was sacrificed for autopsy to see if any accessory glandular tissue present. None found. Total life-span 50 days.

M 2. Young female. Weight 1670 grams. December 7, right adrenal removed. December 13, left adrenal out. Weight 1675 grams. December 14, extract treatment begun; 1.0 cc. given subcutaneously twice daily. January 20, animal in perfect health. Weight 1800 grams. February 1, cat normal. Weight 1904 grams. Sacrificed on this date for autopsy. No accessory gland tissue found. Total life-span 50 days.

M 3. Adult male. Weight 2775 grams. December 3, right adrenal removed. December 13, left adrenal out. Weight 2725 grams. December 14, extract treatment

begun; 1.5 cc. given subcutaneously twice daily. January 19, cat normal. Weight 2800 grams. February 1, cat in excellent health. Animal sacrificed for autopsy. No accessory glands found. Total life-span 50 days.

M 4. Young male. Weight 2175 grams. February 17, right adrenal removed. Weight 2175 grams. February 25, left adrenal out. Weight 2295 grams. February 26, extract treatment begun; 1 cc. twice daily. March 15, cat normal. Weight 2340 grams. April 14, animal died of pneumonia. Had shown no symptoms of adrenal insufficiency. No accessory glands present. Total life-span 47 days.

M 5. Adult male. Weight 3160 grams. December 16, right adrenal removed. December 24, left adrenal removed. Weight 3185 grams. December 25, extract treatment begun; 1.5 cc. given subcutaneously twice daily. January 28, cat normal. Weight 3600 grams. March 30, cat normal. Weight 3820 grams. April 3, cat normal. Weight 3835 grams. Extract discontinued on this date (100 days). Animal in perfect health. April 8, symptoms of adrenal insufficiency noted—anorexia, weakness in hind legs, animal sways when walking. April 10, marked symptoms. Weight 3400 grams. Cat prostrate. April 11, cat died presenting all of the classical symptoms of adrenal insufficiency. Weight 3350 grams. Total life-span 109 days. Weight gain while on extract 650 grams. Weight loss after discontinuing extract 485 grams.

Autopsy: Much fat present in omenta and around kidneys. Thymus greatly enlarged. Liver and spleen congested, pancreas moderately so. No blood in lumen of gut. Heart flaccid and empty, great veins full. Bile present in intestinal tract.

M 6. Adult female. Weight 2130 grams. December 16, right adrenal removed. December 24, left adrenal removed. Weight 2270 grams. December 25, extract treatment begun; 1.5 cc. given twice daily subcutaneously. December 27, animal aborted kittens. Refused food for two days. December 29, resumed eating. Animal normal. March 30, cat normal. Weight 2620 grams. April 3, cat normal. Weight 2850 grams. Extract discontinued (100 days) on this date. White rat placed in cage, cat immediately killed and ate it. April 12, cat showed symptoms of adrenal insufficiency. April 16, cat prostrate. Died 11:30 p.m. Weight 2380 grams. Total life-span 113 days. Weight gain on extract 580 grams. Weight loss after extract discontinued 470 grams.

Autopsy: Marked congestion of liver and spleen, pancreas slightly congested. Kidneys normal. Gut normal. Much fat in omentum. Thymus enlarged. Heart flaccid and empty. Lungs normal.

M 7. Adult male. Weight 3245 grams. February 6, right gland removed. February 22, L. gland out. Weight 2810 grams. Animal had severe respiratory infection for week previous to operation. Refused all food. April 21, cat normal. Weight 3310 grams. May 28, cat normal. Weight 3420 grams. June 5, cat normal. Weight 3420 grams. Extract discontinued on this date. June 13, cat shows distinct symptoms of adrenal insufficiency—anorexia, weakness, etc. June 15, cat prostrate. Died 10 p.m. Weight 2850 grams. Total life-span 112 days. Weight gain while on extract 610 grams. Weight loss after discontinuing extract 570 grams.

Autopsy: Liver and spleen congested, pancreas only slightly so. Much fat in omentum and around kidneys. Gut normal. Enormously enlarged thymus. Lungs and heart normal. One small duodenal ulcer.

M 8. Adult female. Weight 2675 grams. February 5, animal aborted kittens. February 11, R. gland removed. February 17, L. gland removed, weight 2700 grams; 1.5 cc. given subcutaneously twice daily. March 30, cat normal. Weight 3255 grams. May 5, cat normal. Weight 3325 grams. May 27, cat perfectly normal. Weight 3376 grams. Extract discontinued on this date. June 15, cat losing weight

rapidly, but eats. No symptoms. Weight 3150 grams. July 14, cat eats, no symptoms but weight loss great. Weight 2675 grams. July 26, adrenal insufficiency symptoms noted. July 30, animal very weak—rectal temperature 94°, weight 2325, July 31, prostration. Death. Weight 2300 grams. Total life-span 163 days. Weight gain while on extract 676 grams. Weight loss after discontinuing extract 1076 grams.

Autopsy findings: Congestion of liver and spleen, pancreas moderately congested. Thymus greatly enlarged. Thyroid normal. Heart flaccid and empty. Veins distended. Lungs and kidneys normal. No blood in lumen of gut. Careful search revealed no vestiges of accessory cortical tissue.

M 9. Given in the text.

M 10. Adult male. March 8, R. adrenal removed. March 15, L. adrenal removed. Weight 2835 grams; 1.5 cc. given subcutaneously twice daily. April 28, cat normal. Weight 3545 grams. June 23, cat normal. Weight 3400 grams. Extract discontinued. July 5, cat shows symptoms adrenal insufficiency, anorexia, weakness. Weight 2910 grams. July 8, prostration, death. Weight 2750 grams. Total life span 115 days. Weight gain while on extract treatment 565 grams. Weight loss after discontinuing extract 650 grams. This animal developed several abscesses at site of injections. They were drained and healed rapidly. Animal also developed a very severe cold with symptoms of pneumonia, but recovered after two weeks. Suffered a severe weight loss during the interval of the infection.

Autopsy findings: Lungs and kidneys normal. Thymus greatly enlarged. Thyroid normal. No blood in lumen of gut. Heart empty and flaccid. Veins moderately full. Liver and spleen congested. Fat present in omentum and around kidneys.

M 11. Adult male. Weight 2915 grams. April 1, R. gland removed. April 7, L. adrenal removed. Weight 2905 grams; 1.5 cc. given twice daily subcutaneously. May 5, cat normal. Weight 3425 grams. June 2, cat normal. Weight 3680 grams. July 15, cat perfectly normal. Weight 4090 grams. Extract treatment discontinued on this date. July 25, first symptoms noted. Weight 3650 grams. August 10, cat prostrate. Died of adrenal insufficiency at 11 a.m. Weight 3425 grams. Total life span 125 days. Weight gain while on extract treatment 1085 grams. Weight loss after discontinuing extract, 665 grams.

Autopsy findings: Heart flaccid and empty. Great veins distended, much pericardial fat. Thymus greatly enlarged. Large axillary lymph masses. Lung normal. Several small gastric ulcers, one large healed ulcer in pyloric antrum. Much bile in gut tract. Mesenteric lymph nodes enlarged. No blood in lumen of gut. Liver much congested, also spleen. Pancreas normal. Much fat in omentum and around kidneys.

M 12. Young male. Weight 2155 grams. R. gland removed May 25. L. gland out June 2. Weight 2150 grams. Animal not treated, but allowed to develop very severe adrenal insufficiency symptoms. Weight June 13, 1880 grams. Cat normal. Treated with a modified extract. Animal quickly returned to normal. June 16, cat perfectly normal. Weight 1915 grams. July 28, cat normal. Weight 2370 grams. Found dead in cage, morning of July 27. Animal had evidently died in a fit. Animal had eaten heartily previous evening. Had never presented any symptoms of adrenal insufficiency since brought back to normal. Total life span 56 days. Total weight gain while on extract 220 grams. No weight loss. This animal was permitted to develop symptoms of adrenal insufficiency before the extract treatment began.

Autopsy findings: Thymus enlarged, liver, spleen and pancreas normal. Lungs, heart, and kidneys normal. No accessory glands present. Cause of death unknown.

M 13. Adult male. Weight 2700 grams. April 1, R. gland removed. April 10,

L. gland removed. Weight 2720 grams; 1.5 cc. given subcutaneously twice daily. May 10, cat normal. Weight 3103 grams. June 7, cat normal. Weight 2862 grams. July 19, cat perfectly normal. Weight 3231 grams. Extract discontinued. July 26, cat shows symptoms adrenal insufficiency, anorexia, lethargy, slight leg weakness July 30, cat prostrate. Death at 10:15 p.m. Weight 2715 grams. Total life-span 111 days. Weight gain while on extract 511 grams. Weight loss after discontinuing extract 516 grams.

Autopsy findings: Thymus much enlarged. Lymph nodes in mesenteries enlarged. Heart flaccid and empty. Veins full. Lungs normal. Kidneys normal. Liver and spleen congested. Pancreas moderately congested. Much fat in omentum and around kidneys. No blood in lumen of gut. Much bile present. Several healed abscesses present at site of injection area in skin.

M 14. Adult male. Weight 2900 grams. April 1, R. gland removed. April 8, L. gland removed. Weight 3100 grams; 1.5 cc. given subcutaneously twice daily. May 8, cat normal. Weight 3222 grams. June 15, cat normal. Weight 3315 grams. July 17, cat perfectly normal. Weight 3540 grams. Extract discontinued on this date. July 28, cat shows first symptoms. Refuses food, lethargy. Weight 3192 grams. August 4, cat prostrate. Death at 2 p.m. Weight 2973 grams. Total life span 118 days. Weight gain on extract 440 grams. Weight loss after discontinuing extract 567 grams.

Autopsy findings: Thymus enlarged. Lungs and kidneys normal. Liver and spleen congested. Pancreas moderately congested. Gastro-intestinal tract contained much bile. No blood in lumen of gut. Fat present in omentum and around kidneys. Several healed abscesses under skin at site of injection. Small healed gastric ulcers.

M 15. Adult male. Weight 3320 grams. R. gland removed May 16. L. gland removed May 26. Weight 3330 grams; 1.5 cc. given twice daily subcutaneously. June 30, cat normal. Weight 3245 grams. July 8, cat has developed severe infection at site of injections. Treated daily. Weight 3135 grams. July 23, infection has spread over wide area. Animal has developed severe cold. Cat killed on this date. Weight 3020 grams. No accessory glands present. Total life-span 58 days.

Autopsy findings: Severe generalized infection. No accessory glands found.

M 16. Adult male. April 18, R. gland removed. April 28, L. gland removed. Weight 2630 grams; 1.5 cc. extract subcutaneously twice daily. May 26, weight 3105 grams. Cat normal. June 30, weight 3350 grams, cat normal. August 5, weight 3350 grams. Animal normal. Extract discontinued 100 days. August 20, first symptoms of adrenal insufficiency. Slight leg weakness. Cat eats, however. August 22, very severe symptoms. August 23, cat prostrate. Died at 5 p.m. Weight 2850 grams. Total life-span 117 days. Weight gain on extract 720 grams. Weight loss after discontinuing extract 500 grams.

Autopsy: Liver and spleen congested. Heart, lungs, kidneys normal. Extremely large thymus which weighed 8.5 grams. Mesenteric lymph nodes much enlarged, pancreas congested. Stomach and intestinal tract filled with bile. Pyloric end of stomach and duodenum had large number of fresh ulcers present. One ulcer about size of ten cent piece at pyloric end of stomach, along with 8 others, ranging from 2 to 4 mm. in diameter. Two small ulcers in fundic portion of stomach. Six small ulcers on duodenum.

M 17. Female adult. Weight 3001 grams. R. gland removed May 17. L. gland out May 28. Weight 3020 grams; 1.5 cc. twice daily subcutaneously. June 30, cat normal. Weight 3190. July 15, cat normal. Weight 3340 grams. July 17, severe generalized infection developed from injections. July 21, cat killed. No symptoms of adrenal insufficiency. No accessory glands present.

M 18. Adult male. Weight 3780 grams. R. gland removed May 6. L. gland removed May 16. Weight 3730 grams; 1.5 cc. extract twice daily subcutaneously. June 30, cat normal. Weight 3195 grams. Cat had very severe cold. July 13, cat normal. Weight 3825 grams. August 24, cat normal. Weight 4021 grams. Extract discontinued this date. September 5, first symptoms noted. Anorexia, weakness. Weight 3827 grams. September 11, cat prostrate. Died of adrenal insufficiency. Weight 3641 grams. Total life-span 117 days. Weight gain on extract treatment 291 grams. Weight loss after discontinuing extract 380 grams.

Autopsy findings: Thymus enlarged. Heart, lungs and kidneys normal. Liver, spleen and pancreas congested. No blood in lumen of gut. Much bile present. Healed abscess scars at site of injections. No accessory cortical tissue present.

M 19. Adult male. Weight 3240 grams. R. gland removed April 10. L. gland removed April 18. Weight 3215 grams; 1.5 cc. twice daily subcutaneously. May 29, cat normal. Weight 3410 grams. June 25, normal, weight 3507 grams. July 27, cat normal in every respect. Weight 3639. Extract discontinued on this date. August 10, first symptoms noted. Anorexia, lethargy, slight weakness. August 16, cat prostrate. Died 11 a.m. of adrenal insufficiency. Weight 3103 grams. Total life span 120 days. Weight gain on extract treatment 424 grams. Weight loss after extract discontinued, 536 grams.

Autopsy findings: Enlarged thymus. Heart, lungs, kidneys and pancreas normal. Liver and spleen congested. Bile in gut. No blood in lumen of gut. Old healed gastric ulcer. Much fat in omentum and around kidneys. No accessory cortical tissue present.

PROTOCOL OF LONG SURVIVAL CAT WITH ACCESSORY CORTICAL TISSUE. *M X.* Young female. Weight 2180 grams. R. gland removed March 18. L. gland removed March 24. Weight 2215 grams; 1.5 cc. twice daily subcutaneously. April 28, cat normal. Weight 2660 grams. May 26, cat normal. Weight 2700 grams. July 2, cat normal. Weight 2655 grams. Extract discontinued on this date. August 18, weight 2670 grams. Normal in every respect. Animal killed for autopsy. Accessory cortical gland found attached to left renal vein. Gland of considerable size. Microscopic examination showed it to consist only of cortical tissue. Animal remained in perfect condition without weight loss for 47 days following withdrawal of extract. Compare protocol of this animal with that of M 8, with regard to weight loss after discontinuing the extract treatment.

STUDIES ON THE ADRENAL CORTEX

III. The Revival of Cats Prostrate from Adrenal Insufficiency with an Aqueous Extract of the Cortex[1]

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In two earlier papers of this series (This Journal) the writers described the preparation of an active aqueous extract of the adrenal cortex and its effect in maintaining the life of bilaterally adrenalectomized cats. It was demonstrated that such animals when treated with subcutaneous injections of the extract survive indefinitely or at any rate for so long as tested, and remain in normal health. Following cessation of the extract treatment, the animals soon die, exhibiting all of the classical symptoms of adrenal insufficiency.

The type of extract employed in the experiments concerned with long survival (study II of this series) contained appreciable quantities of adrenalin, 0.17 to 0.37 mgm. per cc. When used in small doses such as 0.5 to 1 cc. per kilogram of body weight subcutaneously, it gave excellent results, and the animals remained normal. Extract containing this amount of adrenalin is unsuitable for intravenous or intraperitoneal injection since a small quantity gives rise to adrenalin overdosage symptoms.

However, the original aqueous extract which was used in the long survival experiments (paper II) proved useful in alleviating mild symptoms of adrenal insufficiency—this despite the adrenalin content and the necessity of administering the material subcutaneously (Swingle and Pfiffner, 1930). The protocols of several animals so treated are given below. It may be added at this point that this same type of extract was used successfully when administered subcutaneously, by Dr. L. G. Rowntree of the Mayo Clinic, in the crisis of Addison's disease. (Rowntree, Kintner and Lymburner, 1930.)

The cats were operated, fed and cared for as described in study I of this series of papers, except that the bilaterally adrenalectomized cats were not given extract, but were permitted to develop mild symptoms of adrenal

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insufficiency, such as anorexia, weakness in legs when walking, so that the cat swayed unsteadily from side to side, weight loss and falling body temperature. When the symptoms were quite clear and definite, the extract treatment was begun. All injections in this series of cats were subcutaneous and in no case did an animal receive more than 6 cc. of extract per day. The following protocols summarize the data.

Protocol 1. Adult male. Weight 2775 grams. R. adrenal removed January 3. L. gland removed January 13. Weight 2725 grams. January 14–20, cat normal. Ate heartily. January 21, animal refused food. January 22, symptoms of adrenal insufficiency present. Cat refused food. Showed weakness in hind legs. Swayed from side to side when forced to move about. Very lethargic. Weight 2375 grams. Cat injected subcutaneously 9 a.m., January 22, with 2 cc. of extract. Injection repeated again at 1 p.m. and 10 p.m. January 23, cat about same. Injected extract as on day previous. January 24, cat brighter, interested in its surroundings. Drank some milk in a.m. Ate salmon in p.m. Injected as previously. January 25, cat returning to normal. Ate liver, drank milk and walked about without symptoms. January 26, cat normal in every way. Body temperature normal 101-2°. Weight 2447 grams. From this date on given 1.5 cc. of extract twice daily. Cat remained in normal health and steadily gained weight. February 20, extract was discontinued. Weight 2880 grams. February 27, cat died presenting typical symptoms of adrenal insufficiency.

Protocol 2. Adult male. Weight 2915 grams. R. adrenal removed January 1. Left adrenal removed January 7. Weight 2905 grams. January 8–12, cat normal in every way. January 13, refused food. January 14, weakness in legs apparent. Animal staggered sideways when walking. Refused to move unless prodded. Weight 2730 grams. January 15–16–17, cat given 2 cc. extract subcutaneously three times daily. January 16, weight 2660 grams. January 17, cat resumed eating. Ate liver, drank milk. Walked about without symptoms. January 18, cat followed one around crying for food. No symptoms. Weight 2709 grams. Dosage reduced to 1.5 cc. extract twice daily this date. January 20, cat normal. Weight 2821 grams. This animal was kept on extract treatment (1.5 cc. twice daily) for 100 days and then allowed to die of adrenal insufficiency symptoms after discontinuing the extract. At time of cessation of treatment cat weighed over 4000 grams.

We were eager to test the extract upon untreated cats prostrate, and on the verge of death from adrenal insufficiency, but owing to the adrenalin, were unable to use the original material on cats presenting such severe symptoms. It became necessary, therefore, to devise a method for the removal of practically all of the adrenalin, without diminishing thereby the potency of the extract.

Further fractionation studies were undertaken with the primary object of separating adrenalin from the cortical hormone, and with the secondary object of removing inert material, especially contaminating pigment substances. Several different methods of fractionation, including saponification and ether-acid purification were tried. At this time we wish to present a very simple, but effective method of fractionation which in our hands has given very consistent results, both from the standpoint of yield and purity of product. The cortical extract obtained by this method

is suitable for subcutaneous, intraperitoneal or intravenous use. A preliminary account of this work was made some time ago (Swingle and Pfiffner, 1930a).

The fractionation step is based upon the observation of Whitehorn (1923) that adrenalin is removed quantitatively from solution, by passing the solution through permutit. We have found permutit to be a very valuable reagent for removing adrenalin and much inert material including most of the pigment from the active adrenal cortex fraction. Alcohol was found to be the most convenient solvent. Using the technique described below, the cortical hormone is apparently recovered quantitatively.

The details of the method of fractionation are best illustrated by citing the following typical experiment:

The 70 per cent alcohol-soluble fraction containing the cortical hormone obtained from three kilograms of fresh beef cortex (using the scheme of fractionation described in paper II of this series, THIS JOURNAL) served as the starting material. This solution contained 1.49 gram of solids including 36 mgm. of adrenalin. The solvents were removed by distillation in partial *vacuo* at an external temperature of 45–50° C. Toward the end of the distillation, small quantities (about 30 to 50 cc.) of absolute ethyl alcohol were added to facilitate the removal of most of the water. The residue was taken up in 100 cc. of 95 per cent ethyl alcohol, in which it was completely soluble, and the solution was filtered through two 30-gram portions of permutit, at the rate of about 1 to 2 drops per second. The apparatus used for this purpose consisted of 2 cm. glass tubing of convenient lengths. Each tube was fitted at one end with a one-hole rubber stopper, through which was inserted a short piece of 6 mm. glass tubing. The rate of flow was controlled with a short piece of gum rubber tubing and a Hofmann clamp. The two filtering tubes were placed one above the other. The filtering tube was prepared as follows: a small pledget of cotton was placed in the tube immediately above the stopper. The permutit was poured in, lightly tamped and washed by drawing 100 cc. of 95 per cent ethyl alcohol slowly through the filter. The excess alcohol was removed by suction. After the alcoholic solution of the active fraction had entered the filter, the permutit was washed with one 100 cc. and one 300 cc. portion of 95 per cent ethyl alcohol which was allowed to pass through the filters at about the same rate as the alcoholic solution of the active fraction. The flow was controlled so that the level of wash alcohol in the filtering tube was a few centimeters above the level of the permutit. A gentle suction was required at the end of the washing.

The alcoholic filtrate contained 0.41 gram of solids including less than 1 mgm. of adrenalin. Following concentration to about 100 cc. the filtration procedure was repeated, using two 15-gram portions of permutit.

The filtrate contained 0.40 gram of solids, including approximately 0.05 mgm. of adrenalin.

The alcoholic solution was then concentrated to about 100 cc. and 70 cc. of distilled water were added. The amount of water added at this point depends on the concentration of extract desired. The alcohol was removed and the extract diluted to 100 cc. with distilled water. A milky suspension was obtained, which was very rapidly and conveniently clarified and sterilized, by passing through a Seitz filter. The filtered extract was crystal clear and very pale yellow in color. The filtered extract (100 cc.) contained 0.29 gram of solids, including approximately 0.05 mgm. of adrenalin. The pH (quinhydrone) was 5.65. The addition of 0.8 per cent sodium chloride rendered the extract isotonic. One cubic centimeter represented 30 grams of fresh beef adrenal cortex.

In order to make the results reported in this paper comparable to those reported in papers I and II of this series, THIS JOURNAL, all the extracts used were made up so that 1 cc. represented 30 grams of fresh adrenal cortex.

Since the 3 kilograms of adrenal cortex contained 6.6 grams of adrenalin, and the finished extract only about 0.05 mgm., we feel that for practical purposes the removal of adrenalin can be considered quantitative. We should say at this point that in interpreting assay data, we have assumed for convenience that the cortical hormone *per se* has no effect on blood pressure.

The extracts were stored in the refrigerator, and were generally used within two weeks following their preparation. In one case, however, the extract was stored for three weeks before using, with no detectable deterioration. (See protocol 4.) The effect of preservatives on the cortical hormone is being studied at the present time and the results will be reported at a later date.

One batch of extract was prepared from adrenal cortical tissue which had been stored in alcohol for two weeks at room temperature. The benzene soluble fraction was subsequently stored in the refrigerator in benzene for seven weeks. The finished extract was found to be just as potent as extracts prepared in a minimum period of time. (See protocol 4.) This is additional evidence to support our earlier statements that the cortical hormone is stable in alcohol or benzene solution.

The active fraction which was purified by a single filtration through permutit (20 grams of permutit per kilogram of cortex) will for convenience be designated fraction A. Material which was purified with an additional filtration through permutit (10 grams of permutit per kilogram of cortex) will be referred to as fraction B.

On the basis of the positive results obtained with the active fraction following two filtrations, with permutit, it seems reasonable to assume that the final trace of adrenalin (0.05 mgm.) could be removed with an-

other filtration or two without affecting the cortical hormone. In this study, however, we were concerned primarily with the preparation of an active extract suitable for intravenous use, and two filtrations under the conditions described earlier in this paper have been found ample.

According to bio-assay (blood pressure of dogs) fraction A contained less than 0.01 mgm. of adrenalin per cc., and proved to be excellent material for either subcutaneous or intraperitoneal use.

The adrenalectomized cats were allowed to develop very severe symptoms of adrenal insufficiency before injection. They were prostrate and unable to walk, and if placed upon their feet, promptly collapsed. Death was imminent in all cases. The following protocols give the details of the experiments.

Protocol 3. Adult female. Weight 2721 grams. Right adrenal removed May 5. Left adrenal removed May 12. Weight 2730 grams. May 13–20, cat remained normal. May 21, first symptoms of adrenal insufficiency noted. Cat refused food. Was very lethargic. May 22, weakness in hind limbs when moving about noticeable. May 23–25, animal steadily declining. Very weak. May 26, cat prostrate at 9 a.m. Could not stand erect, but fell upon side when placed upon feet. Rectal temperature 93°. Weight 2460 grams. Body cold and clammy. Given 7 cc. of fraction A intraperitoneally at 10:40 a.m. Given 7 cc. again at 4 p.m. and at 8 p.m. Rectal temperature 93° at 8:20 p.m. Cat in very bad condition. May 27, 8 a.m., cat up walking about. Weakness of legs present. Animal attempted to eat some salmon but gave up. Drank few mouthfuls of milk. Given 7 cc. extract intraperitoneally at 9 a.m. and again at 7 p.m. Cat much brighter but still weak. Rectal temperature 96.2°. May 28, 9:30 a.m., cat seemed normal. Ate liver and drank milk. Weakness in legs had disappeared, but cat was not lively. Rectal temperature 100.2° at 8 p.m. May 29. Given 7 cc. extract at 10 a.m. Ate voraciously. Ran about in normal fashion. Rectal temperature 101.4°. No symptoms of adrenal insufficiency. May 30–June 6, cat given 2 cc. fraction A subcutaneously daily. Animal normal in every way. Weight 2748 grams. June 6, rectal temperature 101.4° June 7, extract discontinued. June 13, cat died with typical symptoms of adrenal insufficiency. Weight 2402 grams. Rectal temperature 94°.

Autopsy: Liver and spleen congested. Pancreas, lungs and kidneys normal. Heart empty, great veins filled. Thymus somewhat enlarged. Gut tract normal. Small duodenal ulcer present. No accessory cortical tissue found.

Protocol 4. Adult male. Weight 2672 grams. R. gland removed May 5. L. gland removed May 12. Weight 2690 grams. May 13–17, cat normal. Ate. May 18, cat "droopy." Refused all food. May 19, 8 a.m., cat very weak. Staggered about in semi-circles when attempting to walk. 12 m., cat prostrate and unable to rise to feet. Involuntary emission of urine and feces; verging on coma. Rectal temperature 92°. Cat in terminal stages of adrenal insufficiency and barely alive. Body cold and clammy. Given 10 cc. fraction A intraperitoneally at 2:10 p.m. At 3:30 p.m., cat up walking about, but very weak. At 3:50, cat placed in cage. It stood up on hind legs with fore paws on the wire attempting to get out. Animal looked 100 per cent improved. 8 p.m., cat given 10 cc. intraperitoneally. Rectal temperature 100.3°. Weight 2310 grams. May 20, 9 a.m., cat looked normal. Drank milk and ate liver eagerly. Walked about in normal fashion, but showed slight signs of weakness in hind legs. Injected three times with 5 cc. at each injection. Rectal

temperature 101.2°. May 21, cat perfectly normal. No symptoms. Animal ate heartily and played with string when dangled in front of him. Weight 2437 grams. Rectal temperature 101.5 at 8 a.m. Extract discontinued upon this date. May 22–26, cat remained in normal condition and ate heartily. May 27, animal again presented symptoms of adrenal insufficiency. Died exhibiting typical symptoms May 31, 9:40 a.m.

Autopsy: Liver and spleen congested. Pancreas slightly congested. Heart flaccid and empty. Great veins moderately filled. Lungs and kidneys normal. Gut tract normal. Thymus normal. No accessory cortical tissue present.

Protocol 5. Adult male. Weight 3605 grams. R. gland removed May 18. L. gland removed May 24. Weight 3582 grams. May 25–June 7, cat remained normal. Ate heartily. June 8, first symptoms of adrenal insufficiency. Noted anorexia, lethargy and leg weakness. June 9, cat very bad condition. Rectal temperature 94°. Cat unable to stand or walk. Weight 3240 grams. Five cubic centimeters fraction A injected intraperitoneally three times during day. 10 p.m., no change in animal's condition. Rectal temperature 94° at 10:10 p.m. June 10, 8:30 a.m., cat much better. Sat up and could walk about, but weak. Given same dose of extract as on previous day. At 2:30 p.m., animal ate large hunks of fresh liver and drank milk. Rectal temperature 100.1°. June 11, animal in excellent condition, ate, ran about and presented no symptoms of adrenal insufficiency. Weight 3360 grams. Rectal temperature 102°. Extract discontinued on this date. June 12–15, cat remained normal and ate hearily. June 16, 9 a.m., cat found in coma. Rectal temperature 93°. Body cold and clammy. Given three 6 cc. injections intraperitoneally during day. 11 p.m., cat greatly improved. Rectal temperature 98.2°. June 17, cat again normal. No symptoms present. Ate liver and drank milk. At 8:20 a.m., rectal temperature 101.8°. Extract treatment discontinued this date. June 18–22, cat normal. Marked symptoms appeared June 23. Animal died 10:42 p.m., June 24, exhibiting all the classical symptoms of adrenal insufficiency.

Autopsy: Liver, spleen and pancreas congested. Heart, lungs and kidneys normal. Gut tract normal but some congestions noted. Thymus probably slightly enlarged. No accessory cortical tissue present.

Protocol 6. Adult female. Weight 3122 grams. R. gland removed June 20. L. gland removed June 28. Weight 3073 grams. June 28–July 5, cat normal. July 6, first symptoms of adrenal insufficiency noted. Anorexia and weakness in hind limbs. July 7, symptoms marked. July 10, cat prostrate and in coma at 8:40 a.m. Rectal temperature 93.1°. Weight 2701 grams. Injected three times during day intraperitoneally with 8 cc. of extract. July 11, cat much improved. Sat up and drank milk. Was weak when walking. Rectal temperature 97.3°. Given 8 cc. intraperitoneally at 9 a.m. At 2:10 p.m., cat ate large quantity of liver. Injected with 5 cc. at 8 p.m. July 12, cat normal. No symptoms present. Animal ate liver and drank milk. Ran about the laboratory and was very active. Rectal temperature 101.6 at 9 a.m. Weight 2821 grams. Extract discontinued on this date. Cat remained normal and ate heartily until July 16 when adrenal insufficiency symptoms again appeared. Cat died July 18 at 1:40 p.m. Weight 2520 grams.

Autopsy: Liver and spleen congested. Pancreas moderately congested. Heart, lungs and kidneys normal. Gut tract filled with bile. Thymus normal or possibly somewhat enlarged.

Protocol 7. Young male. R. gland removed May 25. L. gland removed June 2. Weight 2155 grams. June 2–8, cat normal. June 9, cat presented first symptoms of adrenal insufficiency. Anorexia, lethargy and weakness in legs when walking. June 10, cat exhibited severe symptoms, but could still stand erect and move about. Staggers about when walking. June 11, cat in prostration at 9 a.m. Weight 1700

grams. Rectal temperature 94°. Animal injected *subcutaneously* four times during day with 5 cc. of fraction A. June 12, cat much improved but refused all food. Given four injections of 5 cc. each subcutaneously during the day. At 4 p.m. cat drank some milk and ate a few bites of salmon. June 13, cat excellent condition. Ate liver and drank milk. Rectal temperature 101.3°. Weight 1880 grams. On this date the injections with fraction A were stopped and the animal placed on the original aqueous fraction (study II) receiving subcutaneously 1 cc. twice daily. This animal remained normal and free from symptoms from June 13–July 28. On this date cat was at the peak of its weight—2320 grams and ate heartily. Cat was found dead in cage at 8 a.m. July 29. Cause of death unknown. Autopsy revealed no sign of accessory tissue.

Protocol 8. Adult male. R. gland removed March 4. Weight 3815 grams. L. gland removed March 11. Weight 3710 grams. March 12–16, cat normal. March 17, cat refused all food. Weakness in hind legs apparent. Animal staggered about when walking. Injected with 5 cc. of fraction A *subcutaneously*. March 18, cat ate salmon, but appetite poor. Injected twice this date with 3 cc. extract subcutaneously. March 19, cat ate meat and drank milk. Cat in good condition, but somewhat lethargic. Weight 3200 grams. March 20, same treatment continued. March 20-22, cat normal. On latter date fraction A discontinued and original aqueous extract given twice daily subcutaneously, 1.5 cc. at an injection. This animal remained in normal condition for 100 days, when the extract treatment was discontinued, and died within eight days after cessation of extract treatment.

The protocols of the animals given above show conclusively that fraction A remains highly active and potent after a single filtration through permutit, which reduces the adrenalin content to less than 0.01 mgm. per cc. Animals verging on death from adrenal insufficiency can be restored to normal condition without symptoms within 72 hours following the first intraperitoneal injection of extract. Moreover, the data in protocol 5 demonstrate that comatose animals can be restored to normal repeatedly. There appears to be no reason, so far as we know at present, why adrenalectomized cats prostrate with symptoms cannot be used again and again in testing the potency of an extract. When symptoms disappear after treating, the cats cannot be distinguished from normal unoperated animals. Protocols 7 and 8 also show that subcutaneous injections of fraction A are effective in restoring the animals to normal condition, following the onset of severe symptoms, and that such animals (protocol 8) can be kept free from all symptoms for long periods by subcutaneous injections.

Several of the cats when injected with relatively large doses of fraction A (10 cc.) showed some evanescent symptoms of adrenalin overdosage. The symptoms were slight, but nevertheless apparent. These symptoms did not appear in the experiments where fraction B was employed.

Fraction B, as shown by bio-assay (blood pressure of dogs) contains but a minute amount of adrenalin—between 1–1,500,000 and 1–2,000,000. In other respects this fraction did not differ from A. When tested upon cats, prostrate with adrenal insufficiency, it was found to be very active physiologically as the following protocols indicate.

Protocol 9. Adult male. Weight 3864 grams. R. adrenal removed June 25. L. gland removed July 1. Weight 3810 grams. Cat remained normal from July 2–11. First symptoms of adrenal insufficiency noted July 12 at 9 a.m. Animal refused food, was droopy and showed weakness when walking. July 13, symptoms marked. July 14, cat prostrate at 11:10 a.m. Rectal temperature 94°. Weight 3471 grams. Body cold and clammy. Injected three times during day, with 5 cc. of fraction B intra-

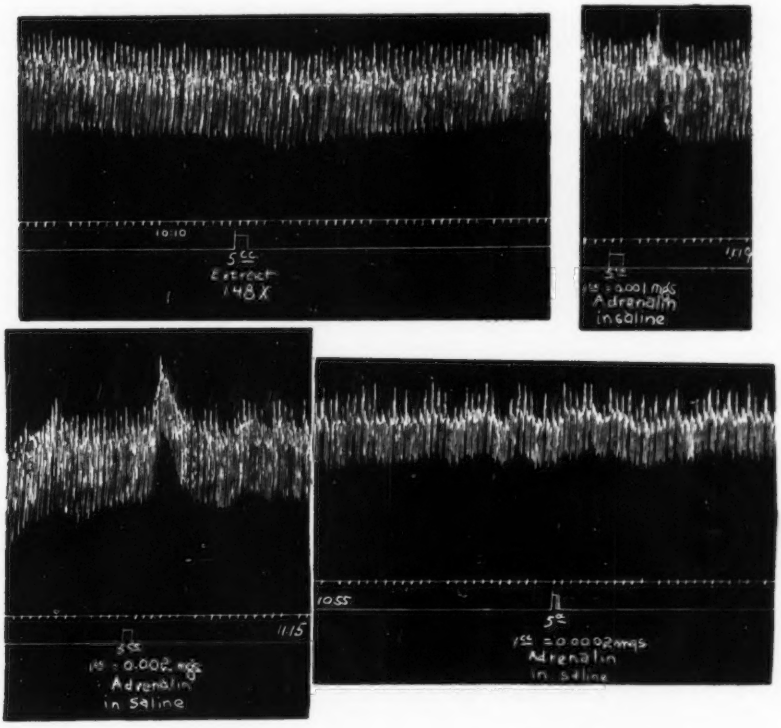

Fig. 1. Effect of fraction B upon blood pressure. Dog. Male. Weight 8 kgm. Morphine-ether anesthesia. Nonatropinized and with vagi intact. Time interval 5 seconds. One cubic centimeter of extract employed equivalent to 30 grams of adrenal cortex. Five cubic centimeters of solution injected in all cases.

peritoneally. 4 p.m., cat ate large quantity liver. 6 p.m., cat drank 35 cc. of milk. Slight weakness in hind limbs when walking only symptom present. Injected 5 cc. fraction B at 10 p.m. July 16, cat crying for food at 8:10 a.m. Ate liver voraciously and in large quantities. No symptoms of adrenal insufficiency present. Rectal temperature 101.2°. Weight 3612 grams. Extract discontinued on this date. Cat remained free from symptoms until July 21, when they again appeared. Animal died of adrenal insufficiency July 24 at 5:44 p.m. Weight 3443 grams.

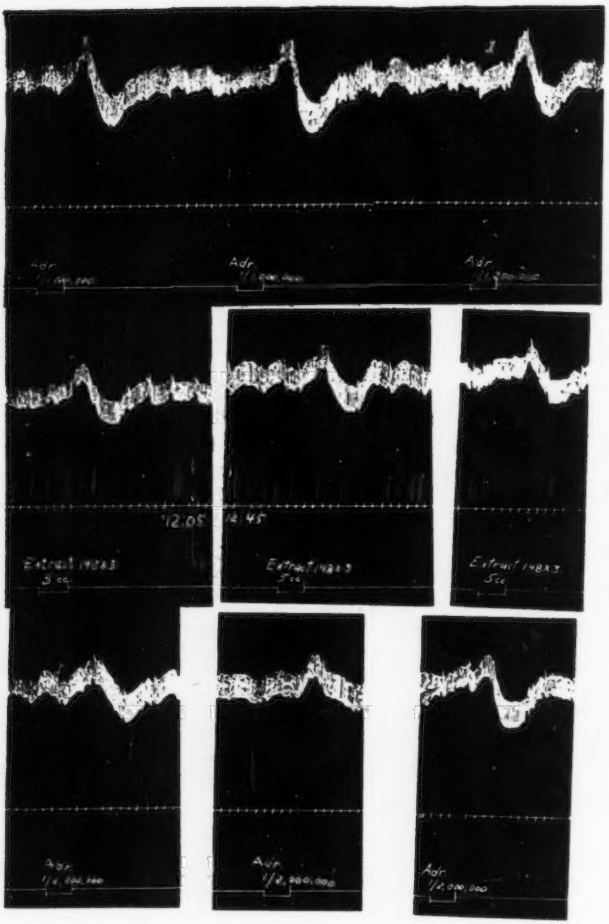

Fig. 2. Effect of fraction B upon blood pressure. Dog. Male. Weight 10 kgm. Morphine-ether anesthesia. Atropinized and with vagi cut. Time interval 5 seconds. One cubic centimeter of extract employed equivalent to 30 grams adrenal cortex. Five cubic centimeters of solution injected in all cases. Adrenalin content approximately 1–2,000,000.

Autopsy: Liver and spleen congested. Pancreas, heart, kidneys and lungs normal. Gut tract normal. Thymus possibly slightly enlarged. No accessory cortical tissue present.

Protocol 10. Adult male. Weight 2432 grams. R. adrenal removed August 26. Weight 2421 grams. Rectal temperature 101.6°. August 27–September 2, cat remained normal and ate heartily. No symptoms. September 3, cat showed first symptoms such as anorexia and leg weakness. Weight 2206 grams. Animal staggered about when walking. Rectal temperature 97.8°. September 4, cat exhibited very marked symptoms of adrenal insufficiency. Very weak. Lay upon side. Body cold. Rectal temperature 3 p.m., 94°. Weight 2124 grams. September 5, 9 a.m. Cat so weak it could not stand. Lies prostrate. Rectal temperature 93°. Body cold and clammy. Weight 2106 grams. Injected 4 cc. fraction B intravenously at 10 a.m. At 1:10 p.m. cat drank 30 cc. milk. Climbed upon a box about 18 inches high. Rectal temperature at 2:15 was 94°. Injected intravenously with 3 cc. extract at 7 p.m. September 6 at 9 a.m. cat much brighter. Rectal temperature 100°. Leg weakness greatly improved. Cat walked about. Injected 3 cc. extract intravenously at 10:45 a.m., at 5 p.m. cat drank milk and ate few bites of liver. Injected 3 cc. extract intravenously at 7 p.m. September 7 at 9 a.m. cat whining for food. Drank milk and ate liver; walked about in good condition. Rectal temperature 101.6°, cat normal, purred and rubbed against his cage. Sharpened his claws, whined for food. Weight 2207 grams. Injected 5 cc. extract intravenously at 9:40 a.m. September 8, cat ran about laboratory whining for food. Ate large quantity liver and drank bowl of milk. When animal placed in cage with a bad tempered male the cats engaged in a hearty fight. Rectal temperature 101.6°. Weight 2346 grams. Animal perfectly normal.

September 9. Cat normal, no treatment. Ate liver, etc.

September 10. Cat normal, no treatment. Ate liver, etc. Weight 2471 grams.

September 11. Cat normal, no treatment. Ate liver and salmon.

September 12. Cat normal, no treatment. Ate liver. Not so lively in a.m.

September 15. Cat showed weakness, staggered about. No appetite, weight 2302 grams. Rectal temperature 96.5°.

September 16. Marked symptoms. Rectal temperature 94° at 9:30 a.m.

September 17. Cat died during night. Autopsy showed nothing unusual.

The experiments discussed in detail in the protocols show beyond any doubt, that animals prostrate and on the verge of death from adrenal insufficiency can be restored to normal health by treatment with either fraction A or fraction B. Those animals receiving fraction B also demonstrate that adrenalin is quite unnecessary as a part of the extract in order for the latter to maintain its activity in the treatment of adrenal insufficiency. This fraction contains less than 1–1,500,000 adrenalin, and this quantity is too small to be of any significance physiologically in the terminal stages of adrenal insufficiency.

It is interesting to note that large doses of fraction B can be given without deleterious effects upon the animal. A two-kilogram adrenalectomized male cat presenting symptoms was injected intraperitorially with 12 cc. of fraction B. This dose was followed within four hours with another intraperitoneal injection of 10 cc., and this again with 10 cc. three hours later, making a total of 32 cc. of the extract within 12 hours. All symp-

toms of adrenal insufficiency disappeared, and the cat remained in normal condition. There is certainly nothing toxic in the extract so far as we are able to judge from the behaviour of cats so treated.

SUMMARY AND CONCLUSIONS

A method is described for the preparation of a potent extract of beef adrenal cortex, suitable for subcutaneous, intraperitoneal and intravenous use. It is practically free of adrenalin. When the extract is made up so that one cubic centimeter represents 30 grams of fresh adrenal cortex, the solid content is about 0.3 per cent and the adrenalin content about one part in two million.

This extract is sufficiently potent to make possible complete adrenal cortex replacement therapy in acute adrenal insufficiency in experimental animals (cats).

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METABOLIC STUDIES DURING PREGNANCY AND MENSTRUATION

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In 1924, data were reported on the heat production, in a normal woman before conception, during pregnancy and the puerperium and for four months after the cessation of lactation and the reëstablishment of menstruation, a total period of seventeen months. In agreement with several other observers, our study showed that there is an increase in the total energy production of the normal pregnant woman during the latter part of pregnancy; although in our case it was not demonstrable before the eighth month as no observations were made in the seventh month; in the last month the total calories for each hour were 25 per cent greater than before conception. However, if the total heat production for each hour was divided by the sum of the surface areas of the mother and of the fetus, no significant increase in heat production was observed. Therefore, it appeared that the heat production of a unit mass of the tissue of the mother probably remained unchanged and that the increased metabolism was due to an increase in the quantity of active protoplasmic tissue, consisting largely of the fetal tissues and in lesser part of the maternal structures. In the previous paper reports of a similar nature which were available in the literature also were reviewed and in the main they confirmed our own study. Detailed reports have not appeared since then.

The subject of our first study again became pregnant two and a half years later and we were able to observe her somewhat more closely during this latter pregnancy (her fifth) at which time she was aged thirty-eight years. The last menstrual period was November 2, 1926, and parturition took place August 9, 1927, making a period of gestation of approximately forty weeks' duration. The subject was in good health during the entire pregnancy. The infant weighed 3.4 kgm. at birth and the placenta weighed 600 grams.

For the determination of the basal metabolic rates the subject came to the laboratory without breakfast and rested half an hour before the mask was adjusted to collect the expired air in a gasometer. Duplicate samples of the expired air were analyzed on the Haldane apparatus. The technic

of the gasometer method as described by Boothby and Sandiford was followed in detail. The subject was well trained in this test so that from this standpoint the determinations were satisfactory in every way. During the 280 days of pregnancy duplicate and frequently triplicate determinations were made on eighty-four days although none was made during the ninth lunar month. For periods of approximately ten days each during the fourth, fifth, seventh, eighth and tenth lunar months of pregnancy and immediately after delivery the subject was on a diet accurately measured in the diet kitchen of our laboratory. Daily specimens of urine were collected for each period and toward the end of each period an analysis of the blood was made. Unfortunately, it was impossible to collect the feces.

For the analysis of the blood, the methods of Folin were used to determine the urea, creatinine, non-protein nitrogen, uric acid, sugar and amino-acid; the total nitrogen was determined by the micro method of Kjeldahl; chlorides by the method of Whitehorn; the inorganic phosphate by the method of Bell and Doisy; calcium, potassium and sodium by the method of Kramer and Tisdall and magnesium by a combination of the methods of Kramer and Tisdall and of Bell and Doisy; total base determination was done by the method of Stadie and Ross. For the urinalysis total nitrogen was done by the method of Kjeldahl; Folin's methods were used for urea, ammonia, preformed creatinine, amino-acid, uric acid, total acidity, total sulphate and inorganic sulphate; Benedict's methods were used for total creatinine and total sulphur; inorganic phosphate was done by the method of Bell and Doisy; chlorides by the method of Volhard; total base by the method of Fisk; for the determination of calcium, potassium and sodium, the methods of Kramer and Tisdall were employed, and finally for the magnesium a combination of the methods of Kramer and Tisdall and of Bell and Doisy was used. Atwater and Bryant's tables were used to calculate the protein, fat and carbohydrate in the diet and Sherman's tables for the inorganic salts in the food.

In figure 1 are plotted the total calories for each hour (curve D), the calories for each square meter calculated by the usual method (curve C^1) and the calories for each square meter calculated by the method outlined in our previous paper, allowing for the surface area of the mother plus the surface area of the fetus (curve C); the basal metabolic rate was calculated in the ordinary manner, using the DuBois standards (curve B^1) and also was calculated on the basis of the combined surface areas of the mother and of the fetus, using the DuBois standards for the mother (curve B) and finally the calories per kilogram of body weight (curve A). In figure 2 are plotted the averages for each lunar month of the total calories each hour (curve D), the calories for each square meter, calculated by the two methods, (cruves C^1 and C), the basal metabolic rate also calculated by the two methods (curves B^1 and B) and finally the calories per kilogram of body

weight (curve *A*). The solid dots represent the data of our first study and the circles represent the average figures for this report. The similarity of the curves of both studies is striking.

At the end of the third month a gradual increase in the total heat production began which later increased more rapidly and reached the highest point

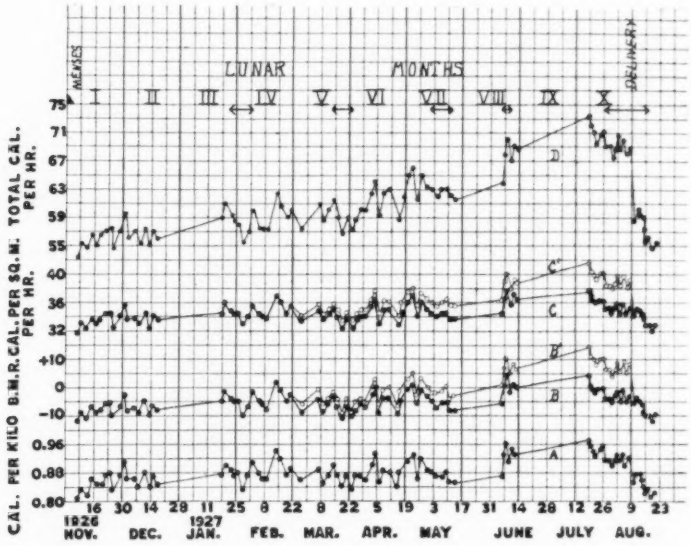

Fig. 1. Heat production during pregnancy and for nine days after delivery. Subject, aged thirty-eight years, height 160 cm. Curve *A* represents the calories for each kilogram of body weight. Curve *B* represents the basal metabolic rate calculated during the course of pregnancy by dividing the total calories each hour by the sum of the surface area of the mother and fetus, and comparing the result obtained with the DuBois normal standards, for the mother, 36.5 calories. Curve B^1 represents the basal metabolic rate calculated by the usual method, using the DuBois surface area and normal standards. Curve *C* represents the calories for each square meter each hour derived for the course of pregnancy as described for curve *B*. Curve C^1 represents the calories for each square meter each hour, obtained by dividing the total calories each hour by the DuBois surface area obtained by using the total weight of mother and fetus in the usual manner. Curve *D* represents the total calories for each hour.

just before delivery. This increase occurred much earlier in this study than in the first study, in which a definite increase was not shown until the eighth month, no observation being made during the seventh month. In both pregnancies the total heat production just before delivery had increased approximately 25 per cent and had reached almost exactly the same

level. Fetal movements probably affect individual determinations somewhat; if anything, these movements were apparently thought by the mother, to be greater during the tests of the first experiment than of the second, although this must be considered of little significance as it is a subjective observation. On the day of delivery the subject's weight had increased by 12 per cent and the calories for each kilogram had increased by 9

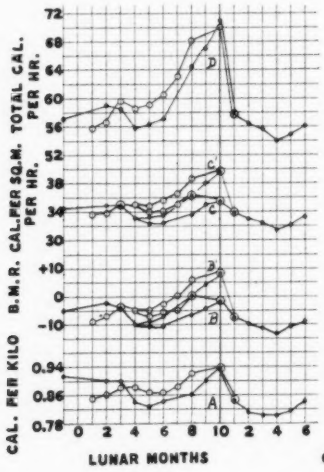

Fig. 2

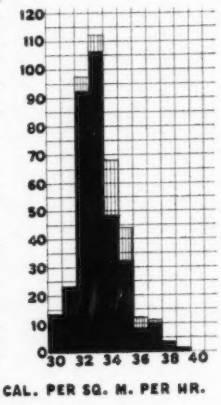

Fig. 3

Fig. 2. Heat production during pregnancy averaged according to lunar months, in the same subject. Curves A, B, B^1, C, C^1 and D represent the average figures for each lunar month of the corresponding curves A, B, B^1, C, C^1 and D in figure 1. The data of the first study are plotted as solid circles and the data of the second study as open circles.

Fig. 3. Basal heat production of the subject plotted as calories per square meter per hour. The curve shows the frequency of distribution around the mean of 33.1 calories per square meter per hour. The solid black spaces represent a total of 336 determinations when the subject was not pregnant, and the perpendicular lines represent the forty-six determinations made during the first three months of both pregnancies.

per cent. The basal metabolic rate in the morning after delivery was −6 per cent as compared with the average rate of −9 per cent for the first lunar month.

Since the infant weighed 3.4 kgm. at birth it is possible to estimate for the various fetal ages the weight of the fetus and to obtain its surface area by using the chart previously published by Sandiford. If the total calories for each hour are divided by the surface area of the mother based on her

weight less the estimated weight of the fetus, plus the surface area of the fetus, the calories per square meter per hour show variations that are well within the daily variations of a normal subject, which confirms the observation previously made by Sandiford and Wheeler. Furthermore, if the calories per square meter per hour for three days after confinement (34.7) are multiplied by the surface area based on the weight of the mother less the weight of the fetus for four days before confinement (1.74), the total calories per hour for the mother alone are obtained (60.4). If from the average of the total calories per hour for four days before confinement (68.9) are subtracted the total calories of the mother alone (60.4) as calculated above, the total calories of the fetus and placenta are obtained (8.5). As the placenta is approximately 15 per cent of the total weight of fetus and placenta and as the nearest approximation that can be made of the relative metabolism of each is on the basis of weight, we can calculate that the heat production of the fetus alone is not far from 7.2 calories; this divided by the surface area of the fetus (0.23) gives the heat production of the latter as 31 calories per square meter per hour, which is within the normal values found by Talbot of approximately 27 to 33 calories for each square meter for newborn infants.

Besides the foregoing observations we have made 336 observations on the subject when she was not pregnant and which extended over a period from February, 1923, to December, 1929, while she was between the age of 34 and 41 years. During this period when not pregnant her weight varied from 62 to 71 kgm. Her height is 160 cm. The data are plotted as calories per square meter per hour in solid black on figure 3 and in addition the determinations (46) during the first three months of both pregnancies are superimposed as the perpendicular lines. Considering only those values in solid black, the curve so produced shows the frequency of distribution around the mean of 33.1 $\pm$ 0.06 calories per square meter hourly with a standard deviation of 1.56 $\pm$ 0.04 calories and that 73 per cent of the determinations are within 1 calorie, or 3 per cent, of the mean and all determinations are within -3 and $+$ 6 calories. This curve of frequency distribution shows a skewness in that there is a tendency for the accidental variation from the average to be more frequent and larger on the high than on the low side. This is the usual experience in basal metabolism determinations, as any disturbance or upset on the part of the subject will cause an elevation of the rate and there are no corresponding causes to produce a lower rate.

A recent article of Benedict and Finn reviews the literature on the effect of menstruation on the heat production. While obtaining the normal data on our subject we made observations during fourteen different menstrual periods; these data are plotted in figure 4 as calories per square meter per hour, as well as a curve representing the average of all the periods. In ten

periods there seemed to be a decided tendency toward a slightly lowered heat production during the menstrual period, preceded by a rise in the premenstrual period and with a gradual rise in the postmenstrual period to the higher values obtained in the premenstrual period; our curves are similar to those of Benedict and Finn. However, as originally shown by Zuntz, such variations are small. In fact, if we average all the determinations for the fourteen menstrual periods for the week preceding menstruation, the week of menstruation and for each of the two following weeks,[1] the calories per square meter are, respectively, 33.3 $\pm$ 0.2, 32.5 $\pm$ 0.3, 32.9 $\pm$ 0.2 and 33.0 $\pm$ 0.3. The standard deviation for each group is 1.2, 1.5, 0.9 and 1.5. The difference between the premenstrual and the men-

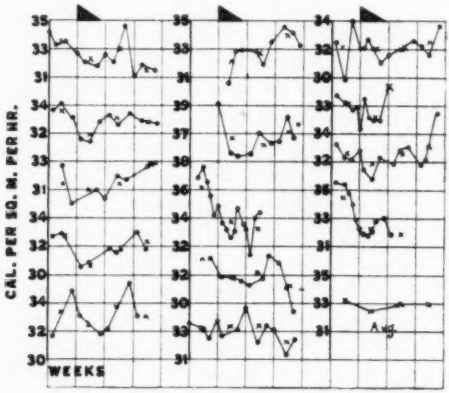

Fig. 4. Heat production before, during and after menstruation plotted as calories per square meter per hour. The solid dots represent the individual determinations and the crosses represent the weekly average figures of these determinations for each menstrual period. The weekly averages of all the determinations before, during and for the first and second weeks after the fourteen menstruation periods are plotted as the curve in the lower rigth hand corner.

strual period is 0.8 calorie with a probable error of $\pm$ 0.4 calories, which indicates that the observed difference has 9 to 1 chances of being statistically significant. Even if this difference is a true difference it does not necessarily indicate a change in the intensity of the oxidation processes per se as they are within the range produced by variations in the degree of physical and mental relaxation. We wish to emphasize that variations of this magnitude can readily be due to varying degrees of relaxation dependent both on the mental status and the physical discomfort of the subject;

[1] No essentially different figures are obtained if the values are grouped into five day instead of seven day periods.

the time relationship of these factors to the menstrual period, as well as their intensity, can readily vary in different subjects. Consequently we can see no evidence in our data that menstruation or an uncomplicated

TABLE 1

Nitrogen partition products in urine

	WEEK OF PREGNANCY										AFTER DELIVERY	
	13		20		27		32		38 to 40			
	Grams	Per cent of total nitrogen	Grams	Per cent of total nitrogen	Grams	Per cent of total nitrogen	Grams	Per cent of total nitrogen	Grams	Per cent of total nitrogen	Grams	Per cent of total nitrogen
Food N	11.2		14.1		14.7		14.9		14.5		13.8	
Total N	11.91		12.19		12.43		12.35		11.99		12.46	
Urea N	9.78	82.1	9.84	80.7	9.72	78.2	9.52	77.1	9.52	79.4	9.94	79.8
Ammonia N	0.42	3.53	0.50	4.10	0.48	3.86	0.52	4.21	0.52	4.34	0.41	3.29
Total creatinine N	0.68	5.71	0.72	5.91	0.76	6.11	0.72	5.83	0.60	5.00	0.57	4.57
Preformed creatinine N	0.63	5.29	0.60	4.92	0.62	4.99	0.62	5.02	0.47*	3.92	0.45*	3.61
Creatine N	0.05	0.42	0.12	0.99	0.14	1.13	0.10	0.81	0.13	1.09	0.12	0.96
Uric acid N	0.17	1.43	0.26	2.13	0.32	2.57	0.36	2.91	0.38	3.17	0.29	2.33
Amino-acid N	0.18	1.51	0.23	1.89	0.23	1.85	0.24	1.94	0.25	2.09	0.20	1.61
Undetermined N	0.69	5.79	0.64	5.25	0.92	7.40	0.99	8.02	0.72	6.00	1.04	8.35
Nitrogen balance	−1.9		+0.5		+0.8		+1.0		+1.1		0	

* The decrease in creatinine nitrogen suggests the possibility of a slight loss of urine; if so, all the urinary data for these two periods may be 10 to 20 per cent too low.

TABLE 2

Non-nitrogenous products in urine

	WEEK OF PREGNANCY					AFTER DELIVERY
	13	20	27	32	38 to 40	
Total acidity, cc. 0.1 N	276	292	337	349	393	389
Inorganic phosphorus, P, gram	0.80	0.59	0.82	1.10	1.11	1.28
Chlorides, Cl, gram	5.64	6.01	7.22	6.28	4.84	5.08
Total sulphur, S, gram	0.89	0.86	0.90	0.88	0.88	0.91
Total sulphate, S, gram	0.76	0.72	0.73	0.73	0.72	0.76
Inorganic sulphate, S, gram	0.69	0.68	0.68	0.68	0.66	0.70
Ethereal sulphur, S, gram	0.07	0.04	0.05	0.06	0.06	0.06
Neutral sulphur, S, gram	0.12	0.14	0.17	0.14	0.17	0.15
Calcium, gram	0.25	0.20	0.28	0.29	0.33	0.31
Magnesium, gram	0.08	0.07	0.07	0.07	0.08	0.08
Potassium, gram	3.05	2.70	3.03	2.98	3.31	3.40
Sodium, gram	3.37	3.81	4.87	4.06	3.07	3.06
Total base, cc. 0.1 N			2,926	2,888	2,544	2,498

pregnancy in a normal woman causes any alteration in the intensity of the oxidation processes going on in the body.

In tables 1 and 2 are given the average figures obtained by analysis of

the urine for the various periods during which the subject was on a known, accurately weighed diet; observation periods were obtained in the thirteenth, twentieth, twenty-seventh, thirty-second and thirty-eighth to fortieth weeks of pregnancy and for nine days after delivery. The average figures for the intake of food for these periods are given in table 3. Since we were not able to obtain the feces during the corresponding periods, the nitrogen balance has been calculated in the usual way by adding 10 per cent (1.1 to 1.5 grams) of the food nitrogen to the urine nitrogen and subtracting this total from the food nitrogen intake. The error produced by not having the nitrogen data on the feces cannot exceed 0.5 gram and probably averages less than that. With the exception of the thirteenth week, when the subject's nitrogen intake was only 11 grams and the nitrogen balance was −1.9 grams the nitrogen balance was always positive on 14

TABLE 3

Average figures for intake of food

	WEEK OF PREGNANCY					AFTER DELIVERY
	13	20	27	32	38 to 40	
Protein, gram	70	88	92	93	90	86
Fat, gram	107	111	113	113	94	96
Carbohydrate, gram	149	175	178	195	225	246
Total calories	1,893	2,106	2,158	2,232	2,166	2,254
Calcium, gram	0.97	1.12	1.11	1.16	1.12	1.25
Magnesium, gram	0.24	0.26	0.24	0.26	0.30	0.32
Potassium, gram	3.67	3.88	3.40	3.52	4.04	4.15
Sodium, gram	3.82	4.61	4.35	3.79	3.28	2.82
Phosphorus, gram	1.21	1.46	1.49	1.52	1.52	1.54
Chloride, gram	5.89	6.95	6.73	5.83	5.05	4.40
Sulphur, gram	0.86	1.03	1.08	1.10	1.07	1.01

to 15 grams nitrogen in the food. On this intake the total nitrogen output in the urine was constant, being between 11.9 to 12.5 grams and the positive nitrogen balance each day was between 0.5 to 1.1 grams. This is in agreement with other observers that moderate nitrogen gains are easily made in a pregnant subject provided the nitrogen intake is adequate (Murlin, Hoffstrom, Landsberg, Wilson, and Coons and Blunt). Our data do not substantiate the idea that, on an adequate diet, the fetus grows at the expense of the mother nor do they indicate that an excessively large positive nitrogen balance occurs. Balances of 5 to 8 grams daily as found by some observers probably indicate incomplete collection of urine. For instance, an average gain of 5 grams of nitrogen would mean that during the 280 days of pregnancy 1,400 grams of nitrogen would be retained; this would be equivalent to 8.75 kgm. of protein on the dry basis which transferred to the wet weight would mean a gain in weight of 35 kgm., or approximately 75

pounds; this is certainly a gain larger than would be expected even if the subject at the beginning of pregnancy was undernourished and during pregnancy was given the best nourishment possible. In order to meet the needs of the fetus in the formation of new protein tissue there should be a gradual increase in the amount of nitrogen retained so that by the end of pregnancy the positive nitrogen balance should be between 1 and 1.5 grams daily; the total nitrogen retained during the 280 days of pregnancy should be between 100 and 150 grams including that necessary for both the fetus and the accessory structures. In our experiment the data quite closely conform to these theoretic values.

The urea output in each period was nearly constant during the early part of pregnancy at 9.8 and 9.7 grams; during the last two months it decreased slightly to 9.5 grams, causing a decrease of the percentage of total nitrogen as urea nitrogen from 82 per cent to between 77 and 79 per cent; this decrease probably is accounted for in part by nitrogen retention and in part by slight increases in some of the other nitrogen partition products. The ammonia nitrogen increased slightly both in absolute amount and in percentage of the total nitrogen and decreased again after delivery; the increase in ammonia probably is due to its formation while in the bladder, as during the latter months of pregnancy it is well known that frequently there is a considerable amount of residual urine. The total creatinine nitrogen increased somewhat from 0.68 to 0.76 gram during the thirteenth, twentieth, and twenty-seventh weeks and decreased again in the thirty-eighth to fortieth weeks and after delivery. The preformed creatinine nitrogen did not change until the last month of pregnancy, at which time there was a 22 per cent decrease which was maintained in the period following delivery. On account of the well known constancy of creatinine elimination under nearly all conditions the sudden drop in both total and preformed creatinine indicates that probably in addition to the increased amount eliminated as creatine there was a small loss due to a slight incontinence of urine, a common occurrence in multiparas. The main value of this figure is to indicate that if such a loss occurred it was quite small and that, therefore, our data concerning the urine are on the whole nearly correct. The creatine nitrogen increased from 0.05 gram in the thirteenth week to 0.12 gram in the twentieth week and then remained quite constant. The uric acid nitrogen showed a gradual and progressive increase from 0.17 gram to 0.38 gram in the last weeks and decreased to 0.29 gram after delivery. The amino-acid nitrogen was 0.18 gram in the thirteenth week, increased to 0.23 gram in the twentieth week and remained constant at this level until after delivery when it decreased again to 0.20 gram. The undetermined nonprotein nitrogen was, in general, higher during the latter than during the earlier months of pregnancy, but in no case greatly exceeded the variation that might be due to the fact that this figure reflects

the accumulative errors of all the other determinations; it is a valuable control figure, as a negative or an unusually high figure calls attention to inaccuracies, if such occur, in the determination of the various nitrogen fractions. Sugar was not present at any time, and only occasionally the slightest possible trace of albumin was present.

During the periods in which the subject was on a carefully controlled diet the intake of calcium averaged 1 gram and the output in the urine varied between 0.2 and 0.3 gram. Unfortunately it is impossible to calculate the calcium balance from our data. Coons and Blunt have shown wide variations in different subjects in the amount of calcium excreted in

TABLE 4

*Analysis of the blood during pregnancy and after delivery**

	WEEK OF PREGNANCY							AFTER DELIVERY
	13	14	21	28	32	38	40	
Total nitrogen, (P)	1.25		1.20	1.20	1.04	1.03	1.08	1.06
Nonprotein N	26	23	23	24	24	30	28	26
Urea N	14	13	13	13	13	16	16	14
Amino acid N	8.0	6.0	6.0	7.0	6.0	7.0	7.0	7.0
Uric acid N	1.02	1.02	1.02	1.09	1.06	1.16	1.19	1.16
Creatinine N	0.63	0.63	0.48	0.56	0.56	0.48	0.56	0.59
Undetermined N	2.4	2.4	2.5	2.4	3.4	5.4	3.3	3.3
Corpuscles, per cent	31	32	34	33	31	30	32	31
Sugar	96	105	99	99	102	105	105	112
Chlorides, (P)		577	611	577	611	609	561	574
Inorganic phosphate, (S)	3.2	3.2	3.3	3.3	3.3	2.9	4.4	4.1
Calcium, (S)	9.3	9.5	9.6	9.8	9.4	9.5	9.8	10.8
Magnesium, (S)	2.3	2.6	2.4	1.8	2.1	1.7	1.8	1.8
Sodium, (S)	314	328		306	300	297	307	311
Potassium, (S)	17	20	16	18	20	16	18	16

* Analyses were done on whole blood unless marked serum (S) or plasma (P).

the feces. However, the calcium excretion in the urine in our subject showed much less variation than in the cases reported by Coons and Blunt. Bauer, Albright and Aub found the calcium excretion essentially normal during gestation. They pointed out, however, that the calcium of a diet low in calcium is apparently not available for the fetus. In our subject the magnesium intake was between 0.2 and 0.3 gram and the average figure for each period for the output in the urine was practically constant at 0.08 gram, slightly less than in the cases reported by Coons and Blunt. The phosphorus intake was between 1.2 and 1.5 grams, and the output of inorganic phosphorus in the urine was between 0.6 and 1.3 gram. The sulphur eliminated in the urine was remarkably constant throughout pregnancy, being between 0.86 and 0.91 gram on an intake varying between 0.9

and 1.1 gram; there is also a noteworthy constancy in the various sulphur partition products.

Complete analyses of the blood were made at various times during pregnancy and after delivery (table 4). The nonprotein nitrogen, urea nitrogen and uric acid nitrogen were within the normal limits, but showed a slight increase in the last month of pregnancy and were still somewhat elevated for two days after delivery. The creatinine nitrogen and amino-acid nitrogen were unchanged throughout the course of pregnancy. There was, however, a definite increase in the undetermined nonprotein nitrogen in the last month of pregnancy; this possibly is not in excess of the combined errors in the individual determinations. Contrary to the observations of Denis, King and Briggs the percentage of urea nitrogen to total nonprotein nitrogen remained essentially unchanged and ranged between 52 and 57 per cent.

The inorganic salts in the blood serum were normal and remained practically unchanged throughout the course of pregnancy, in agreement with the observations of Denis and King.

SUMMARY

There is a gradual increase in the total heat production during pregnancy, amounting at term to 25 per cent in these two particular studies.

Increase in heat production can be accounted for by the added metabolism of the fetus, placenta and accessory structures and there is no evidence to suggest that there is a change in the actual rate or the intensity of heat production of a unit mass of maternal tissue.

After the nineteenth week of pregnancy on an adequate nitrogen intake of more than 85 grams of protein there is a gradually increasing positive nitrogen balance varying between 0.5 and 1.1 gram daily. There is no evidence that the fetus grows at the expense of the mother's tissue if the diet is adequate nor, on the other hand, is there evidence, in an individual who receives a normal amount of nourishment, of excessive protein retention beyond that needed for the fetus and accessory structures.

Nitrogen retention results in a slight decrease in the percentage of total nitrogen eliminated in the urine as urea. There is also a slight increase in the uric acid and amino-acid nitrogen eliminated which would correspond roughly to the increase in new tissue. There is an increase in creatine elimination and no change in the creatinine until the ninth month, when there is a sudden decrease. The ammonia nitrogen increased slightly.

There are no significant changes in the inorganic substances eliminated in the urine of a normal pregnant woman.

There are no significant changes in the chemistry of the blood during the course of normal pregnancy.

During the menstrual cycle, there are no changes in the heat production which can be considered significant of a fundamental change in the intensity of the oxidation processes intrinsically as they are within the range produced by variation in the degree of mental and physical relaxation.

The mean value of 336 basal determinations of the heat production on a normal woman between her thirty-fourth and forty-first year is 33.1 $\pm$ 0.06 calories.

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SCN CONTRACTURE IN SKELETAL MUSCLE

Further Studies in Permeability of Muscle

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A balanced salt solution is characterized by an equilibrium between the different salts contained in the solution with respect to cell functions, and therefore such a solution is suitable to preserve the physiological functions of cells and tissues for a long time. None of the typical ion effects upon cells is observed in a balanced solution while the least disturbance of its equilibrium calls them forth. These ion effects lead to changes in irritability of nerve or muscle, favor or prevent the diastole or the systole of the heart, etc.; in other words they lead to deviations in the normal functions which are observed in the intact body. It has been found by various investigators that Ringer's solution fulfills all requirements of a balanced solution for the tissues of vertebrates. Therefore when typical ion effects are found in Ringer's solution we are confronted with a new and unexpected physiological phenomenon. Such an effect was observed by Libbrecht and Witanowski in the heart of the frog. After the heart was perfused with a potassium-free Ringer's solution in which it still showed a normal beat, replacement of this solution by common Ringer's solution led to a temporary diastolic standstill. This observation is explained by the assumption that the heart acquired a new equilibrium in the K-free Ringer's solution so that the common Ringer's solution caused a typical K-effect because of its higher K-content. Another case of similar kind in a heart was described by Zwaardemaker and referred by him to a change of radioactive substances contained in Ringer's solution. In these cases the effect of Ringer's solution was due to a disturbance in the *kation equilibrium* of the tissue.

There is described in this paper a similar phenomenon in the striped muscle of a frog which is due to a disturbance in the *anion equilibrium*. The immersion of the sartorius in a Ringer's solution containing NaSCN in a concentration which is *below* that causing contracture, has no direct effect upon the muscle but gives rise to a contracture when this solution is replaced by common Ringer's solution. This phenomenon will be designated as an "indirect SCN contracture."

METHOD. For the most part the experiments were done with the sartorius muscle of the frog. Under favorable conditions indirect contracture could also be obtained with the gastrocnemius, but in general this muscle was less suitable than the sartorius. The experiments reproduced in the tables and figures of this paper were all performed on the sartorius of *Rana esculenta*. The contracture was registered in the usual way, the muscle moving an isotonic lever loaded with 1 gram. Magnification due to the lever was seven-fold. In another series of experiments Bürker's isometric lever was used. All solutions used were isotonic with a 0.66 per cent NaCl solution ($\Delta = -0.39°$). It is important to use fresh frogs. During the last two years about two hundred experiments were performed.

I. *The basic experiment.* The "indirect NaSCN contracture" is illustrated by figure A. At the first + Ringer's solution was replaced by 9 cc. Ringer + 9 cc. NaSCN solution. The muscle remained in this solution for 70 seconds and then (at the second +) this solution was replaced by Ringer's. The muscle immersed in SCN-Ringer's was not affected. But if afterwards the muscle was put into Ringer's solution a remarkable contracture took place: indirect contracture. In very irritable muscles fibrillary contractions were superimposed upon the contracture. The duration of the contracture lasted in general from 20 to 60 seconds. Because the contracture takes place in Ringer's or isotonic NaCl-solution, which normally cannot bring about a contracture of striped muscle, this effect is a new phenomenon.

The next task was to analyze the probable mechanism of indirect contracture and obtain a theoretical basis for further experimentation. The assumption was made that the NaSCN-contracture depends upon a change in the NaSCN concentration in the muscle. In the case of direct contracture an increase in NaSCN concentration may take place, but in the indirect one a decrease. This means that NaSCN probably causes the contracture of the muscle in two ways: either by entering or by leaving the cells of the muscle. If this view is correct NaSCN would belong to the group of "Potentialgiften" (Straub). The effect of these poisons depends upon the velocity with which a substance enters or leaves the cell. On this basis the NaSCN effect can be easily explained. If the NaSCN content of the Ringer's solution is too small to cause a direct contracture even after the muscle has been in the solution some minutes, the NaSCN probably enters the cell but does so too gradually to produce any effect. With longer exposure of the muscle to the NaSCN-Ringer's solution,[1] the permeability of the surface layer of the muscle increases, which may be due to an increase in imbibition. Now, it is known that SCN increases imbibition and numer-

[1] NaSCN-Ringer's means a solution consisting of Ringer's solution plus isotonic NaSCN solution.

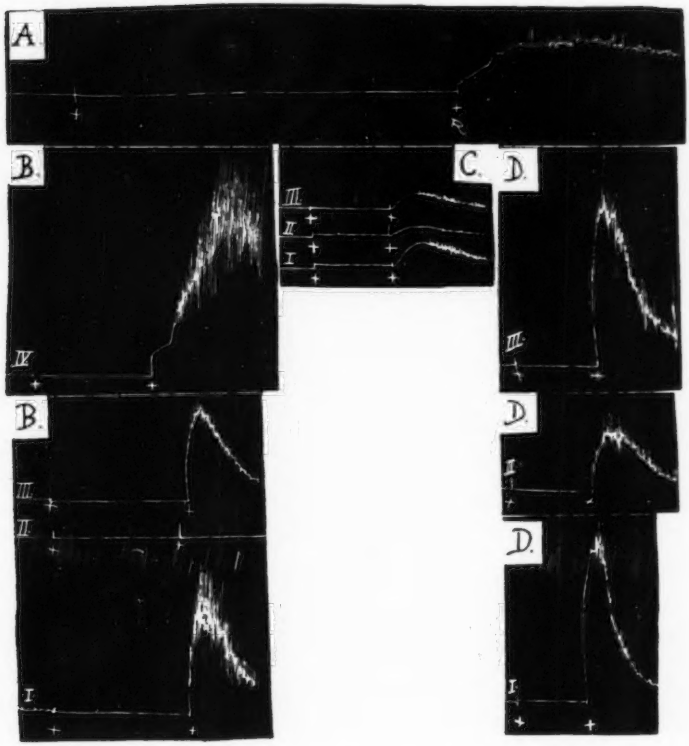

Fig. A. At the first + Ringer's solution was exchanged for 9 cc. isotonic Ringer's + 9 cc. isotonic NaSCN. At the second + this solution was exchanged for 18 cc. Ringer's solution.

Fig. B. At the first + in all experiments of this figure Ringer's was exchanged for 8 cc. Ringer's + 8 cc. NaSCN (isotonic). At the second + this solution was exchanged for: In expt. 1,8 cc. Ringer's + 8 cc. NaCl. In expt. 2,8 cc. Ringer's + 7.5 cc. NaCl + 0.5 cc. $CaCl_2$. In expt. 3,8 cc. Ringer's + 8 cc. NaCl. In expt. 4, 8 cc. Ringer's + 7.5 cc. NaCl + 0.5 cc. $BaCl_2$.

Fig. C. At the first + in all experiments 8 cc. NaSCN + 9 cc. Ringer's. At the second + this solution is exchanged for: In expt. 1 and 3,8 cc. NaCl + 9 cc. Ringer's. In expt. 2, 8 cc. LiCl + 9 cc. Ringer's.

Fig. D. At the first + Ringer's is exchanged for 11 cc. Ringer's + 5 cc. NaSCN. At the second + this solution is exchanged for: In expt. 1 and 3,15 cc. NaCl + 1 cc. m/10 Na_2HPO_4. In expt. 2, 15 cc. NaCl + 1 cc. m/10 NaH_2PO_4.

ous experiments support the conclusion that a close parallelism between imbibition and permeability frequently exists (Gellhorn, 1929). Therefore NaSCN which has entered the cell rather slowly can leave it rapidly if one exchanges the solution for a NaSCN-free Ringer's solution. In this case indirect contracture occurs. It is known from other investigations (for instance, Jendrassik) that the effects of ions, etc., are the same no matter whether there is a drop in concentration from outside to inside or in the opposite sense.

There are two possibilities of proving the correctness of this hypothesis. If the effect in question is SCN contracture it should show the peculiarities of that contracture. This can be tested first by comparison of the isometric tension of the muscle in indirect and in direct NaSCN contracture. Secondly, indirect contracture should be influenced by factors which increase or decrease the permeability. This can be tested in experiments in

TABLE 1

Tension of the sartorius in direct NaSCN-contracture and in tetanus

NUMBER	A TENSION IN THE DIRECT NaSCN CONTRACTURE	B TENSION IN TETANUS AFTER MAXIMAL FARADISATION	$\frac{100 \cdot A}{B}$
	grams	*grams*	*per cent*
1	1	47	2.2
2	1	65	1.5
3	1.5	70	2.2
4	1	68	1.5
5	1	62	1.6
Average			1.8

which the permeability of the muscle is changed either by electrical stimulation of the muscle or by a change in the physico-chemical composition of the solution. That these suppositions are correct is proved by the following experiments.

II. *The isometric tension of the NaSCN contracture.* From the investigations of various authors (Bethe, Gellhorn, Riesser) it is known that the isometric tension of the muscle is less during contracture than during tetanus. This gave a means of comparing the tension of direct and indirect NaSCN contracture. First the tension of the muscle in isotonic NaSCN solution was determined. After an interval of 15 minutes in Ringer's solution, the tension was ascertained after maximal faradic stimuli for 2 seconds. The results are given in table 1.

It is apparent from table 1 that the NaSCN contracture shows very low tensions (on the average 1.8 per cent). The tensions are lower than those found in direct contracture produced by K-salts, acetylcholin, NaOH, etc.

In previous experiments a greater tension was obtained in experiments with KSCN than with KCl for striped (Riesser and Richter) and smooth muscle (Gellhorn, 1928).

The tension of the muscle during indirect contracture is shown in table 2. The muscle when kept for 40 seconds in a solution composed of 12 cc. NaSCN and 5 cc. Ringer's showed no noticeable change in tension. At the end of this time the muscle was immersed in isotonic NaCl solution and indirect contracture occurred, of which the tension was determined. After an interval of 15 minutes in Ringer's solution the tension of the same muscle in tetanus, after faradisation, was measured. The result shows that the tension in the indirect is slightly greater than that in the direct contracture. This was also the case even if the direct and indirect NaSCN contractures were performed on the same muscle. But the order of magnitude is the same and is very characteristic for the SCN-contracture because,

TABLE 2

Tension of the sartorius muscle in the indirect NaSCN contracture and in tetanus

NUMBER	A TENSION IN THE INDIRECT CONTRACTURE	B TENSION AFTER MAXIMAL FARADISATION	$\frac{100 \cdot A}{B}$
	grams	*grams*	*per cent*
1	1.5	60	2.5
2	3.0	54	5.6
3	1.5	70	2.1
4	3.5	60	5.8
5	2.0	58	3.5
Average			3.9

as mentioned above, other contracture-producing substances give far higher values. In the striped muscle the indirect SCN-contracture can also be observed with $Ca(SCN)_2$ or NH_4SCN. In general NaSCN was found to be the most favorable salt for obtaining the indirect SCN-effect. The writer has not as yet been successful in producing an indirect contracture in smooth muscle.

III. *The influence of change in permeability of muscle upon indirect SCN-contracture.* The relation between permeability and indirect SCN contracture is shown by the following experiments which are essentially an application of the author's previous study of the K-contracture of striped muscle. In this study it was shown that the strength of the KCl-contracture could be varied to a great extent by varying the physico-chemical composition of the solution in which the muscle was immersed. It was demonstrated that if, according to the general rules of permeability, the solution caused an increase in permeability, the susceptibility of the muscle

to KCl increased, while a decrease in permeability diminished or suppressed the KCl-contracture. The same laws also hold for indirect NaSCN-contracture. It was first attempted to determine what influence the physicochemical composition of the second solution has, i.e., the one which is substituted for NaSCN-Ringer solution. It was expected that in a pure NaCl solution indirect contracture would be greater than in Ringer's solution. In table 3 an experiment is reproduced in which Ringer's solution and isotonic NaCl were mixed in different ratios and used as the second solution. The influence of these ratios upon indirect contracture was studied. These experiments were performed on the same muscle. In all cases the first solution was the same, consisting of 8 cc. Ringer's solution and 8 cc. isotonic NaSCN solution, and the muscle remained in this solution for 40 seconds. This solution was then exchanged for the second solution, consisting of Ringer's + isotonic NaCl and the indirect contracture was recorded. After every experiment the muscle remained 15 minutes in Ringer's solution. It is evident from table 3 that with increasing ratio $\frac{\text{NaCl}}{\text{Ringer's solution}}$ in the second solution, the height of the indirect SCN-contracture increases. The experiment shows how dependent the phenomenon is upon the permeability of the surface layer of the muscle. It illustrates the well known fact that the permeability in a balanced solution is rather small in comparison with that in a pure NaCl solution.

TABLE 3

Height of the indirect NaSCN-contracture dependent upon the composition of the second solution

First solution: 8 cc. Ringer's + 8 cc. isotonic NaSCN

NUMBER	COMPOSITION OF THE SECOND SOLUTION	HEIGHT OF CONTRACTURE	REMARKS
		mm.	
1	10 cc. Ringer + 6 cc. NaCl (isotonic)	2.5	Very small fibrillary twitches
2	4 cc. Ringer + 12 cc. NaCl	5.0	Fibrillary twitches of moderate size
3	0 cc. Ringer + 16 cc. NaCl	8.0	Very large fibrillary twitches
4	16 cc. Ringer + 0 cc. NaCl	2.2	No fibrillary twitches
5	0 cc. Ringer + 16 cc. NaCl	8.0	Very large fibrillary twitches

Table 3 not only shows the complete reversibility of the phenomenon, but also the influence of the second solution[2] upon the occurrence of fibril-

[2] For the sake of brevity we shall refer to the NaSCN-Ringer as the first solution and to the solution which is NaSCN-free, as the second solution. When the muscle is taken from the first and put into the second solution the indirect contracture takes place.

lary twitches. In Ringer's solution one usually finds very small fibrillary twitches or none at all; but upon the addition of isotonic NaCl these are frequent and are larger the greater the ratio $\frac{\text{NaCl}}{\text{Ringer}}$. Previous authors (Hering, Loeb), observed these identical twitches only when the muscle remained a much longer time in NaCl solution. That the twitches occur immediately in our experiments indicates the great increase in permeability caused by the NaSCN in the first solution.

The substitution of isotonic NaCl for Ringer solution appears to favor indirect contracture by reason of the lack of Ca, the effect of which is to reduce the permeability of the surface layer of the muscle. Thus it was to be expected that an increase in the Ca content of the Ringer's solution would decrease the contracture still more. Further it seemed probable that other bivalent kations would act in the same way. In a preliminary report (Gellhorn, 1930) it was shown that the addition of small amounts of $CaCl_2$, $SrCl_2$ or $MgCl_2$ to Ringer's solution led regularly to a complete suppression of the contracture. But these processes are reversible because succeeding experiments with the same muscle, using Ringer's as a second solution, gave a positive result.

The results of previous experiments on the KCl-contracture and the influence upon its strength of the addition of bivalent kations, suggested that the indirect contracture would be diminished if $BaCl_2$ was added to the second solution. But the experiment showed just the opposite effect. Even very small amounts of $BaCl_2$ strengthened indirect contracture. This is illustrated by figure B. The second tracing shows that the addition of 0.5 cc. isotonic $CaCl_2$ suppresses the indirect contracture. That this effect is reversible is shown in the third tracing in which the control experiment of curve I was repeated with about the same result. Curve IV shows that the equivalent amount of $BaCl_2$ increases indirect contracture very much. This holds not only for the height of the contracture, but also for the magnitude of the fibrillary twitches shown in curve IV.

The increase in indirect contracture caused by adding $BaCl_2$ to the second solution was demonstrated in still another way. If the composition of the second solution is such that no indirect contracture occurs, a small amount of $BaCl_2$ added to the second solution will produce a typical contracture. Thus the Ba acts as if it lowered the threshold of indirect contracture.

On the other hand various monovalent ions influence indirect contracture in a different way if added to the second solution. Figure C shows that Li increases the contracture less than Na does. Further experiments were performed with certain heavy metals and the results again agree with the corresponding experiments done on the KCl-contracture. It was found that the addition of the chlorides of Co or Cu or Fe regularly diminished

or suppressed indirect contracture. The concentrations required to suppress contracture in these experiments were lower than those used in experiments with alkaline earths corresponding to the series: Ca, Sr, Mg, > Cu, > Co, Fe. The effect of Co was easily reversible, but that of Cu and Fe somewhat reduced the susceptibility to NaSCN so that the indirect contracture was diminished a little in the control experiment. It is apparent from table 4 that Co is more effective than Cu, while table 5 shows that the effect of Cu is only partly reversible. Control experiment 3 (table 5) shows a feebler indirect contracture than control experiment 1,

TABLE 4

The influence of heavy metals on indirect contracture

First solution: 8 cc. Ringer + 8 cc. isotonic NaSCN; duration of immersion of the muscle in the first solution, 120 seconds. The experiments in this table were all performed on the same muscle.

NUMBER	COMPOSITION OF THE SECOND SOLUTION	HEIGHT OF CONTRACTURE
		mm.
1	10 cc. Ringer 6 cc. isotonic NaCl	21
2	10 cc. Ringer 5.9 cc. isotonic NaCl 0.1 cc. isotonic $CoCl_2$	12
3	10 cc. Ringer 5.7 cc. isotonic NaCl 0.3 cc. isotonic $CoCl_2$	3.5
4	10 cc. Ringer 6 cc. isotonic NaCl	19
5	10 cc. Ringer 5.7 cc. NaCl 0.3 cc. $CuCl_2$	6.5

but the reactivity of the muscle is still normal because experiment 4 shows the typical increase in contracture in the presence of $BaCl_2$.

Summing up the results it is apparent that the indirect SCN-contracture is increased if the second solution is partially or completely replaced by isotonic NaCl solution. Bivalent ions decrease the permeability and thus if added in small amounts to the second solution decrease the phenomenon. The heavy metals are more effective than the alkaline earths, since even if the former are used in smaller amounts than the latter they still diminish the permeability and in consequence the indirect contracture. The effects described above can be summed up in the series: Ba < Na < Li < Ca, Sr, Mg < Cu < Co, Fe, in which the ions are arranged according to their

inhibitory effect upon direct contracture. There is a close parallelism between these experiments and those concerning the KCl-contracture of the muscle with but one exception: the effect of Ba.

In further experiments the effect of variation of the osmotic pressure and of the pH in the second solution upon indirect contracture was studied. The result showed that indirect contracture, other conditions being equal, decreased with increasing osmotic pressure. It was greater in hypotonic solution than in an isotonic one and was less in a hypertonic one. The influence of pH was studied using phosphate buffer over a pH-range of 6.5 to 8.0. The effect may be illustrated by figure D, which shows that the indirect contracture is stronger in an alkaline solution than in an acid one. The magnitude in a neutral solution is intermediate. The influence of variations in the osmotic pressure and pH is identical in indirect SCN contracture and in KCl contracture.

TABLE 5

Conditions the same as in table 4

NUMBER	COMPOSITION OF THE SECOND SOLUTION	HEIGHT OF CONTRACTURE
		mm.
1	16 cc. NaCl	13.0
2	14 cc. NaCl 2 cc. $CuCl_2$	3.3
3	16 cc. NaCl	6.0
4	15.5 cc. NaCl 0.5 cc. $BaCl_2$	17.0

Further experiments were performed with electrical stimulation. It is known from the investigations of Weiss that stimulation of the muscle leads to an increase in permeability. Thus it was to be expected that indirect contracture would be increased by the application of electrical stimulation. The muscle was accordingly stimulated with submaximal condenser discharge, using the apparatus of Scheminsky. The frequency of stimulation was 40 to 45 per minute. The experiment was done on the same muscle 2 to 4 times alternately with and without stimulation. The composition of the first and second solutions was of course the same in each series of experiments. The result is that under conditions in which an unstimulated muscle gives practically no indirect reaction the phenomenon becomes very distinct if the muscle is stimulated. One can therefore speak of lowering the threshold of the muscle to NaSCN by electrical stimulation.

The hypothesis made above, that indirect contracture is due to the velocity of exosmosis of NaSCN out of the cell is well supported by these

experiments. It is clear that a diminution of the permeability of the surface layer of the muscle leads to a diminution of diffusion and therefore to a diminution or suppression of the phenomenon. On the other hand an increase in permeability must be accompanied by intensification of the phenomenon. This is the case not only if the permeability is altered by changing the physico-chemical composition of the solution, but also if it is altered by applying electrical stimulation. The thorough-going concordance between the experiments with KCl contracture and indirect SCN contracture (with the single exception of the $BaCl_2$ effect) confirms the explanation of the phenomenon on the basis of permeability.

SUMMARY

1. If a Ringer's solution, containing NaSCN in a concentration which is below the threshold for direct contracture, is allowed to bathe the frog sartorius muscle for a definite time, and is then replaced by Ringer's solution, a contracture takes place which is called indirect contracture because of its occurrence in pure Ringer's solution.

2. This indirect contracture can be obtained also by application of other SCN salts such as $Ca(SCN)_2$ and NH_4SCN. This and the observed low value of the isometric tension show that the indirect contracture is a SCN contracture.

3. The strength of the indirect SCN-contracture depends chiefly upon the composition of the second solution which is substituted for SCN-Ringer's solution. It is much greater in NaCl than in Ringer's solution, and by the addition of small amounts of Ca or Sr or Mg or still lower concentrations of heavy metals such as Cu, Co and Fe indirect contracture can be suppressed completely. It increases with decreasing osmotic pressure and increasing pH. It is favored by stimulation of the muscle with condensor discharges. The assumption that the strength of indirect contracture is determined by the velocity with which NaSCN leaves the muscle cell and therefore by the permeability of the muscle easily explains the facts described above. This for the reason that it has been found that ions increase and decrease indirect contracture in the same sense as they influence permeability. There is but one exception: $BaCl_2$ if added to the second solution increases the strength of indirect contracture.

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THE RELATION OF OVARIAN HORMONES TO ELECTRO-MYOGRAPHICALLY DETERMINED ACHILLES REFLEX TIME

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Physiologists and psychologists have observed a relationship between the functional sex glands and both general and specific behavior. Among others, Richter and Wislocki (7) and Richter (6), working on the rat, have shown a perfect correspondence to exist between changes in spontaneous activity and activity of the gonads, as shown by the normal cycle, by castration, or by implantation. Macht and Hyndman (5), by injection of mentoxin, a substance contained in menstrual blood serum, and more recently Tsai (14), by partial and complete castrations, have shown that test animals which have been treated by either procedure are inferior to normal controls in orientation, in maze learning, in maze running, and in solving a single platform box problem.

To our knowledge no one has yet shown whether the difference in behavior between control and experimental animals is due to the effect of a sex hormone on the nervous system, to changes in metabolism, or to some other physiological adjustment which may accompany the experimental conditions. The present paper reports the effect, in the rat, of 1, the normal sex cycle; 2, injections of extracts of follicular fluid and of corpus lúteum, and 3, of pregnancy and of pseudo-pregnancy, on electro-myographically determined Achilles reflex time.

MATERIAL AND METHOD. The recording apparatus has been fully described elsewhere (8). Briefly, it consists of a three-stage resistance-coupled amplifier, an oscillograph, a vacuum tube oscillator and a signal circuit. The oscillograph is manufactured by the Westinghouse Electric and Manufacturing Company. A supersensitive element capable of responding within 1/20,000 of a second was used for recording the electrical changes in the muscle. A special photographic unit was employed which permitted recording on standard Eastman Super-Speed motion picture film. A General Radio Company low frequency oscillator, type 377, was used to furnish a time line of 1,000 complete cycles per second. The

signal circuit was activated by discharging a condenser which previously had been charged.

The electrodes were thin brass strips, 3 mm. by 6 mm., covered with canton flannel soaked in a saturated salt solution. Both electrodes were set in a wooden block about 4 mm. apart.

The stimulus was given with a small metal hammer. When the tendon was struck the contact of the hammer with a small metal strip insulated from, but directly over, the Achilles tendon discharged the signal circuit.

The animals employed were all females, so chosen because of the ease with which the oestrous cycle lent itself to the plan of research. All animals were virginal. Those in the pregnancy and pseudo-pregnancy series were allowed to copulate for the first time for the purpose of this experiment. Vaginal smears were made daily according to the technique of Long and Evans (4) on all animals.

In order to elicit Achilles jerks each animal was placed in a split-hinged metal tube which covered the entire body with the exception of the head and the fore and hind legs. Round the animal's neck was fastened a metal collar. The body covering and the collar were made fast to metal stands which supported the animal about ten inches above the level of the table. All reflex jerks were taken from the left hind leg, which was shaved to insure good contact with the electrodes. The electrodes were placed over the gastrocnemius muscle with the proximal electrode over the head of the muscle. All stimuli probably were maximal, although Tuttle (13) has shown that strength of stimulus is not a determining factor in reflex time, and all determinations were taken as the average of five to seven consecutive jerks.

Experimental. Sixteen normal females were followed through thirty consecutive cycles, varying from one to four cycles per individual, and Achilles reflex time determinations were taken during oestrus and dioestrus on each. The average reflex time during oestrus was found to be 7.6 sigma and the average reflex time during dioestrus 6.1 sigma, the average difference being 1.4 sigma. This difference is, we feel, quite significant since it is almost three times the jerk to jerk variation (0.5 sigma) at one testing of any animal.

In an attempt to demonstrate that the differences in reflex time between oestrus and dioestrus were actually attributable to the action of active sex hormones two further series of experiments were undertaken. In the first, six females were castrated and daily vaginal smears were taken for three weeks to check the operation. The animals then were injected with Estrogen and reflex time determinations were taken when the smear indicated full oestrus, usually two or three days following the final injection. Although the extract utilized was standardized to 25 R.U. per cc. when injected intramuscularly, it was found necessary to inject 3 cc. subcu-

taneously (in 0.5 cc. doses at four hour intervals) in each instance in order to evoke a fairly strong positive reaction. Subcutaneous injections were given since it was thought that those administered intramuscularly were being absorbed and eliminated too rapidly for full oestrous changes to occur. These animals after an interval of a week were then each injected subcutaneously and in the same dosage with 3 cc. of Lipo-lutein[1] and reflex time records taken two days following the final injection.

Control animals received subcutaneous injections of 3 cc. of sterile Ringer's solution in the same dosage, and reflex time records were taken two days following the last injection.

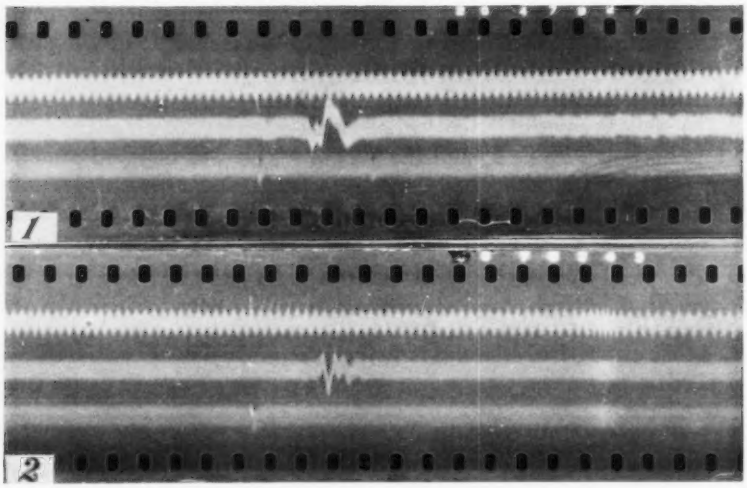

Fig. 1. Achilles reflex time record of dioestrus in rat L1. From the top downward the curves are, respectively, the time line (in 1/1,000 second intervals), the action current line, and the signal line.

Fig. 2. Achilles reflex time record of oestrus in rat L1. The curves are the same as before.

Table 1 summarizes results of the injection experiments. It will be seen that administration of Estrogen, containing the active principle of follicular fluid, lengthened Achilles reflex time, whereas injection of Lipo-lutein, containing the active principle of the corpus luteum, shortened Achilles reflex time. Ringer's solution was without effect. Any difference in reflex time of greater than 0.5 sigma is considered significant, since the range of reflex time at any one testing was never greater than this figure.

[1] Both Estrogen and Lipo-lutein were very kindly furnished by Parke, Davis & Co., Detroit.

TABLE 1

The effect on Achilles reflex time of injections of Estrogen, Lipo-lutein, and Ringer's solution

TYPE OF TREATMENT	ANIMAL					
	CR1	CR-	CR4	CR11	CR3	CR2
Untreated castrate	6.7	6.9	6.7	7.2	6.9	7.0
3 cc. Estrogen	7.9	8.0	8.4	8.2	8.3	7.8
3 cc. Lipo-lutein	6.0	6.0	6.2	6.2	5.9	6.4
3 cc. Ringer's solution	6.6	7.0	6.6	7.0	7.0	7.0

TABLE 2

Achilles reflex time determinations on pregnant animals

DAYS AFTER COPULATION	RAT WR		RAT RI		RAT RV		RAT R-	
	A. R. T.	Vaginal smear	A. R. T.	Vaginal smear	A. R. T.	Vaginal smear	A. R. T.	Vaginal smear
1	7.2	M	7.6	M	7.3	M	6.5	M
3	—	—	7.2	D	6.8	D	6.5	D
5	—	—	7.2	D	6.5	D	5.9	D
6	6.6	D	—	—	—	—	—	—
7	—		7.0	D	6.5	D	5.9	D
8	—		—	—	—	—	—	—
10	6.1	D	—	—	—	—	—	—
11	—		7.0	*	7.0	*	7.0	*
13	—		7.5	D	7.2	D	7.3	D
14	7.0	D	—	—	—	—	—	—
15	—		7.5	D	7.2	D	7.2	D
17	7.1	D	7.5	D	7.2	D	7.7	D
18	—		7.5	D	—	—	7.7	D
19	7.2	D	8.3	D	7.5	D	7.8	D
20	—		—	—	—	—	—	—
21	7.4	E	8.8	E	7.5	D	8.0	D
22					8.0	E	8.3	E
Records following parturition								
HOURS POST-PARTUM								
3	7.5	E	—	—	8.7	E	—	—
5	—	—	8.4	E	—	—	—	—
10	—	—	6.8	D	6.3	D	6.8	M
24	6.4	D	6.8	D	6.3	D	—	—
48	6.1	D	—	—	—	—	6.0	D

* Placental sign.

M, metoestrus; D, dioestrus; P, pro-oestrus; E, oestrus.

In the final series reflex time determinations were taken under conditions which took advantage of the fluctuating concentration of the follicular hormone and the luteal hormone which occurs in the normal course of events in the intact animals. It is well known that following fertile copulation corpora lutea form which remain functional through the major portion of term; should copulation prove sterile, however, they persist for a time only and give rise to a condition of pseudo-pregnancy. It seemed advisable to determine what effect these two periods of luteal dominance would exercise upon reflex time.

TABLE 3

Achilles reflex time determinations on pseudo-pregnant animals

DAYS AFTER COPULATION	RAT HR		RAT L–		RAT LI	
	A. R. T.	Vaginal smear	A. R. T.	Vaginal smear	A. R. T.	Vaginal smear
1	7.8	M	7.6	M	6.8	M
2	—	—	—	—	—	—
3	—	—	6.7	D	6.5	D
4	6.6	D	—	—	—	—
5	—	—	—	—	—	—
6	—	—	6.2	D	6.5	D
7	5.9	D	6.5	D	6.4	D
8	—	—	—	—	—	—
9	—	—	6.5	D	5.6	D
10	6.4	P	—	—	—	—
11	—	—	6.4	D	5.5	D
12	7.2	E	6.4	D	—	—
13	—	—	—	—	—	—
14	—	—	—	—	5.3	P
15	—	—	—	—	—	—
16	—	—	6.1	D	7.3	E
17	—	—	—	—	—	—
18	—	—	7.0	E	—	—

Accordingly, seven animals were mated, three with sterile males. Achilles reflex time determinations included, in addition to observations throughout the term of gestation, the immediate post-partum oestrus and the subsequent dioestrous period. Tables 2 and 3 tabulate the records on these two series of animals. In the pregnant series, a gradual decrease in reflex time will be noted, up until the tenth or eleventh day, after which the time steadily increased until the time of parturition, which was followed by a sudden decrease. The pseudo-pregnant series was characterized by a decrease in time throughout the dioestrous interval, with an increase at the onset of oestrus.

Discussion. All three experiments demonstrated that a lengthened Achilles reflex time accompanied any increase in the concentration of follicular hormone, or its extract, in the system of the animal. Conversely shortened Achilles reflex time was shown to accompany an increase in the concentration of luteal hormone, or its extract.

The lengthening of reflex time commencing with the eleventh day of pregnancy, at a time when the phase of the ovarian cycle is distinctly luteal, may in all probability be associated with the fact that oestrin occurs in progressively increasing quantities in blood and urine during the later stages of gestation (Ascheim and Zondek, 2; Fels, 3; Zondek, 16). Despite the fact that corpus luetum hormone, essential to normal gestation, is present in relatively large quantities during pregnancy, its possible influence on reflex time apparently is overshadowed by that of the steadily increasing oestrin content in the pregnant animal. Further, it is significant that reflex time shortened rapidly following parturition, particularly in view of the observation that the oestrin content of blood and urine diminishes very abruptly at this time (Ascheim and Zondek, 1; Veler and Doisy, 15, and others).

Variability in reflex time has been demonstrated by Travis and Dorsey (9), (10), (11), to be the result of a functional disequilibration in the inhibitory action of higher or lower irradiational nervous centers. This view recently has gained support through the work of Travis and Herren (12), who have shown the cerebral cortex to be a functional part of the Achilles reflex arc. In the present work, the lengthened Achilles reflex time appearing coincident with the liberation (i.e., increase) of follicular hormone indicated a heightened activity of the higher nervous levels. This conclusion is in accord with the findings of Richter and of Tsai, previously cited. The shortened Achilles reflex time appeared coincident with the increase of corpus luteum hormone, and indicted a decrease in the activity of the higher nervous levels which allowed a relatively uninhibited passage of conduction in the lower arcs. This latter conclusion agrees with the report of Macht and Hyndman. Although the authors are not at this time prepared to say whether these hormones act directly on the nervous tissue of the higher levels or through the medium of other nervous connections it is evident that the sex hormones do have an influence on the nervous system and it seems very probable that the reported changes in behavior following changes in the sex cycle or as a result of castration, implantation, and the like, are essentially reflections of changes in tone of the different levels due to the influence of the sex hormone.

SUMMARY

Determinations on sixteen female rats through thirty oestrous cycles showed a long Achilles reflex time during the oestrous period and a short Achilles reflex time during the dioestrous period.

Determinations on castrates indicated a lengthened Achilles reflex time following injection of extract of follicular fluid and a shortened Achilles reflex time consequent to injections of extract of corpus luteum, whereas controls displayed no significant change.

The pregnant animal was characterized by a gradual decrease in Achilles reflex time until the tenth or eleventh day of term, at which time a gradual increase in reflex time appeared, culminating in the extremely long reflex time of parturition.

Pseudo-pregnant animals showed a gradual decrease in Achilles reflex time corresponding in length to the functional life of the corpora lutea of pregnancy.

It is concluded that the sex hormones play a vital part in the interaction of the various neural levels, either through direct influence on nervous tissues of higher levels, or indirectly through other connections with the higher levels.

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THE RÔLE OF THE SALIVARY GLANDS IN THE THIRST MECHANISM

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Two main theories have been advanced to explain the mechanism of the sensation of thirst. According to one, thirst is a general bodily sensation; the other states that the sensation of thirst originates locally in the mouth and pharynx. In his article, "The Physiological Basis of Thirst," Cannon (1) summarizes the early evidence for the two theories.

1. The theory that thirst is a general sensation. According to Schiff, thirst arises as a result of a lowering of the water content of the body. The feeling of dryness in the throat is secondary and the local reference to the pharynx is due to the association of experiences. Evidence commonly cited in support of this theory is the following:

Intravenous injections in dogs and man abolish the desire to drink (Dupuytren, Orfila, Magendie). Claude Bernard noted that a dog with a gastric fistula continued to drink until fatigued. Mayer (2) found that in thirsting animals there was an increase in the osmotic pressure of the blood. His explanation of thirst was that the excitation produced by the blood with abnormal molecular concentration is transmitted (by sensory nerves from the blood vessels) to a medullary center controlling the vascular changes which regulate the osmotic pressure of the blood. The general disturbance of the whole body leads to malaise and a sensation of dryness in the pharynx accompanied by a desire to drink. Wettendorf (3) found that dogs might give evidence of thirst before their blood became hypertonic and that a dog can apparently quench his thirst with normal salt solution without lowering the osmotic pressure of the blood. Wettendorf ascribed the origin of thirst not to changes in blood concentration and their influence upon the central nervous system, but to withdrawal of water from the tissues.

2. The theory that thirst is a sensation of local origin. In 1885 Lepidi-Chioti and Fubini abolished thirst in a patient with marked polyuria by brushing the throat with a weak solution of cocaine, the effect persisting 15 to 35 minutes. Valenti, in 1910, cocainized the upper part of the esophagus of thirsting dogs and noted that they refused drink although still able to swallow.

In seeking to explain the thirst mechanism, Cannon first calls attention to the dryness of the mouth and throat which is emphasized in accounts of severe thirst, such as that given by King (4). The symptoms described were absence of the salivary and mucous secretions, parched mouths and throats, and inability to swallow dry food, as well as dimness of vision, deafness, tottering gait, delirium, dyspnea, and a feeling of suffocation. However, King also states: "The sensibility of the lingual and buccal mucous membranes was so much impaired that they could not perceive when anything was in their mouth," yet one is led to believe that thirst was present to an extreme degree. The importance of the glands of the mouth and pharynx in keeping the mucous membrane moist, and thus preventing thirst, has been asserted by Weber (5), who was of the opinion that a diminution of the water content of the blood might decrease the secretions upon the mucous surfaces and so lead to thirst. Ludwig (6) and Schaefer (7) expressed similar views. Cannon, in particular, has emphasized this view. He advances the theory that the salivary glands are responsive to the state of hydration of the body, and that when, for any reason, they fail to secrete sufficiently, thirst develops, due to local dryness of the mouth and pharynx.

It has been shown that reflex secretion of saliva tends to occur under conditions which cause dryness of the mouth, such as dry food (Pavlov, 8), and breathing with the mouth open (Zebrowski, 9). Cannon, working on himself, induced thirst by sweating and abstaining from water. He found that he produced less saliva when thirsty than when not thirsty, and that the relationship between the salivary flow and the sensation of thirst was definite. Boring (10) made similar experiments, his results agreeing in the main with Cannon's. However, one subject noted the onset of thirst before any marked decrease in the amount of the salivary secretion. Cannon also tried the effect of atropine, and while under its influence, "experienced all the symptoms of thirst." Rinsing the mouth with weak novocaine abolished thirst; he did not drink, and yet thirst disappeared when the atropine effect wore off.

Additionl evidence in support of Cannon's point of view has appeared since his original article was written. Pack (11) deprived rabbits of water for seven days. Profuse salivation was then induced in some of the animals by means of pilocarpine and these animals were observed to drink less than the rabbits which had received no pilocarpine. Gantt (12) has shown that both the unconditioned and the food conditioned reflex secretion of the parotid and submaxillary glands in children is diminished after a period of water deprivation. Crisler (13) reports that in dogs the conditioned reflex salivary secretion developed by morphine was diminished in quantity during periods of dehydration.

Some writers consider marked dryness of the mouth and pharynx as

synonymous with thirst. Cannon (1), Siebeck (14), Meyer (15), but Mueller (16), in particular, assets that dryness of the mouth is a sensation distinct from true thirst. He states that in his experiments on man dryness due to mouth breathing and atropine are not like thirst. Desire for water can be experienced while the mouth is still wet, and mere wetting of the mouth does not give relief. Severe thirst may occur in nephritics and in cardiac patients who have retention of water in the tissues as edema, and be relieved at the onset of diuresis. Wier, Larson, and Rowntree (17) tested out the relationship of dryness of the mouth to thirst in nine patients with diabetes insipidus. They painted the patients' throats with a weak solution of cocaine to the point of anesthesia without being able to control either the polydypsia or the polyuria. Neither did pilocarpine pushed to the point of salivation (in 4 cases) relieve the intense thirst. Three of the nine patients gave a definite history of the onset of thirst before the onset of their polyuria, that is, before any marked loss of fluid from the body had occurred. Wier, Larson and Rowntree, therefore, conclude that in diabetes insipidus thirst is more than a local sensation due to dryness of the mouth and throat.

One finds additional evidence that thirst is not dependent upon the absence of the salivary secretions from cases in which the salivary glands are either congenitally absent, or for some reason have ceased to secrete saliva. The reports of such cases emphasize the very dry condition of the mouth, and the necessity for taking fluids along with the food to facilitate swallowing, but no mention is made of increased desire for water, which should have been present if the salivary glands were truly so important in regulating the water intake. It appeared possible to make a crucial test of the importance of the salivary secretion in the control of water ingestion by completely extirpating the salivary glands. If, indeed, a diminished secretion of saliva leads to local dryness of the mouth, which in turn causes the animal to drink, a total elimination of the salivary secretion should produce an increase in the daily ingestion of water by the animal.

Bidder and Schmidt (quoted by Cannon) tied off the ducts of all the salivary glands in dogs and noted that the layer of fluid on the buccal mucosa was much diminished and that the desire for water was enormously increased. Extirpation of one or more of the salivary glands in animals has been done by a large number of workers. Budge, in 1842, extirpated the parotid, submaxillary and sublingual glands in dogs. However, the dog, in common with all carnivors and rodents, possesses on each side, beneath the zygomatic arch, another mucous gland, the orbital gland, which is about the size of the submaxillary. Fehr (18) was the first to extirpate the salivary glands completely in dogs. He stated that the animals drank a little more water than before, presumably to increase the ease of chewing and swallowing, but he gave no measurements of fluid

intake before and after operation. Minkowski (19) also extirpated all eight glands in dogs, but made no mention of an increased fluid intake after the operation.

METHODS. In these experiments, the supposed relationship of the salivary secretion to the preservation of the moist condition of the oral mucous membranes and to the prevention of thirst was put to a test by extirpation of all eight glands in dogs. The animals were placed upon a diet of raw or partially cooked meat and bread, to which was added water or milk in proportions which will be indicated later in this article. This was done to insure a diet moist enough to avoid the possibility that the animals might drink water merely to wash down their food, as Fehr thought was the case in his experiments. The animals were permitted to drink water as they desired, since the purpose of the experiment was to note any spontaneous development of thirst after extirpation of the salivary glands. The water drunk, the water content of the food, the amount of food eaten, and the urine output were recorded.

The salivary glands were removed in three to four operations. In this way there was only slight disturbance of the usual food and fluid intake. Sometimes the dog would even eat and drink as usual on the day of the operation. The parotid, submaxillary and sublingual glands of one side were removed at one operation. After a recovery period of six to ten days the glands of the opposite side were extirpated. The orbital glands were usually removed one at a time at different operations, since the local swelling resulting from this operation is considerable. However, an orbital gland may conveniently be removed at the same operation with the other salivary glands of the same side. The parotid was dissected out carefully, the facial nerve being preserved. In the case of the orbital gland, removal was effected through an incision through the masseter muscle parallel with and just inferior to the zygomatic arch. This was the approach described and used by Fehr for his original extirpation of the gland. In some instances considerable post-operative swelling was noted, but recovery was rapid.

Six animals were observed in all. For convenience in discussion they are divided into two groups of three dogs each. Of the original group of three animals, two are still under observation one year from the time of the final operation. Although they have been used for other experimental work, observation on their fluid intake has been made from time to time. The third was used for other work and was killed one year from the time of the final operation. The conclusions stated in this article are based chiefly upon the findings in these three animals. About eight months ago, Miss Dorothy Palmer, working in this department, kindly permitted me to observe the fluid intake on three of her dogs whose salivary glands we removed. These will be referred to as group II.

The three male dogs in group I (dogs 3, 4 and 7), weighing 6.3 to 9.1 kgm., were placed on a diet of equal parts of raw or slightly cooked lean meat and baker's bread, and some bone meal. All of them ate greedily at first, but after they had gained in weight, they spontaneously reduced their food intake, and after this had occurred, less food was offered them. With minor variations their food intake remained at this level throughout the period of observation. When they failed to eat all the food offered, the meat was always eaten and the bread refused. Allowance is made for this in the calculation of the water content of the food.

As has been stated, sufficient water was added to the food to make it moist. The proportion was 50 cc. water to 100 grams of food until three weeks after the last operation. After this, the quantity was reduced to 25 cc. water per 100 grams of food. The animals appeared not to mind unmoistened or actually dry bread, except dog 3, who had very poor teeth. As a control measure, nine months after the last operation, dogs 3 and 4 were placed on the same food intake they had had in the preoperative period, and the daily fluid intake was again noted.

Results. Observation of the animals used in this experiment over a period of one year has led me to believe that one can get very nearly objective evidence about thirst in dogs. There appears to be little of the psychic element in their drinking. When conditions remain fairly constant, and the dogs are well, this fluid intake varies within the same range month after month, the daily fluctuations being considerable in some animals and small in others. As a rule, the dogs used in this experiment showed no tendency to drink simply because water was offered. This observation was interpreted to mean that they drank because they were thirsty and not from habit.

The accompanying chart shows the fluid intake of dog 4 at intervals over a period of 12 months. Five day averages are recorded and the days of operation have been omitted from the calculations. The observations are typical for the dogs of group I. The water intake of all three dogs showed wide daily variations within certain definite limits. In all, there was a much lower fluid intake in the period immediately following the last operation than in the pre-operative period. A part of this decrease can no doubt be regarded as a direct result of the diminished food intake, but not all of it is to be so considered. To test this point, nine months after the last operation dogs 3 and 4 were again placed on the quantity of food eaten in the pre-operative period, but their fluid intake did not quite return to the pre-operative level. In none of these dogs was there any evidence of an increased desire for water.

The dogs in group II had been used for determinations of basal metabolism, had reached a constant weight, and ate nearly constant amounts of meat and bread with 250 cc. of milk daily. The pre-operative and post-

operative periods of observation were short in this group, but the results are entirely in agreement with the observations on group I. Dog 13, female, drank a remarkably constant amount of water daily. Dog 14, male, showed wide daily fluctuations in intake, but drank the same average quantity of water five months after operation as during the periods between operations. On the other hand, dog 15, male, drank considerably less five months after operation than before, although his food intake had been somewhat increased.

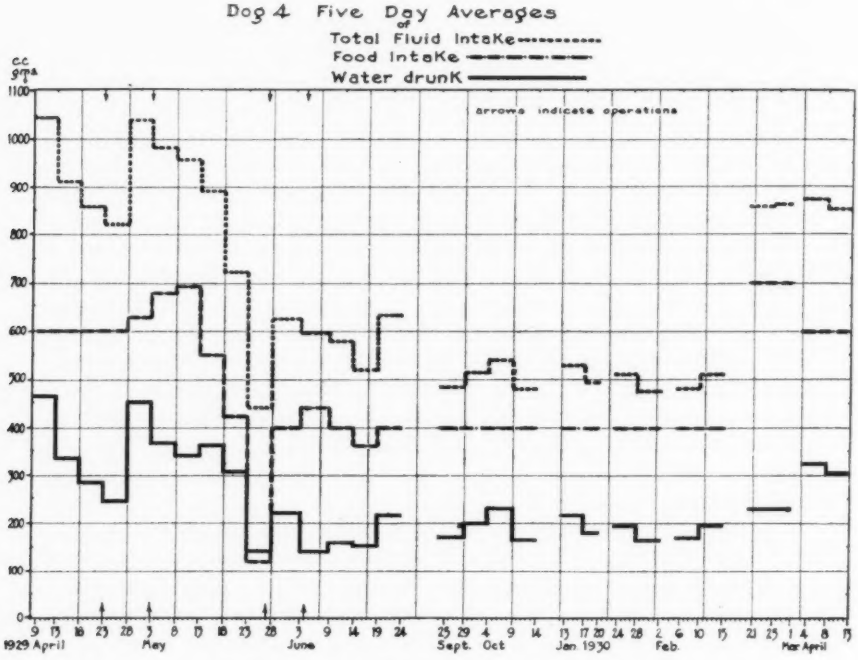

Fig. 1

Five of the six dogs are alive and in good condition. Dog 7 was used for other work and then killed. A point of interest is the remarkably moist condition of the mucous membrane of the mouth which can exist in the entire absence of the salivary secretion. The mucosa of lips, cheeks and pharynx has remained entirely normal. The tongues of all the dogs show a tendency to fissuring, which is most evident in the case of dog 4. However, since the mucous membrane of the tongue is in all cases intact, the condition appears to cause no discomfort.

The quantity of secretion poured out by the glands of the buccal, pharyngeal and nasal mucosa has been measured on dog 7, by collection from an esophageal fistula. When the dog was lying quietly the secretion averaged 8.9 grams of extremely viscid mucus per hour. During sleep the quantity fell as low as 1 gram per hour. Five milligrams of pilocarpine, given subcutaneously, caused a secretion of slightly more than 20 grams in the first half hour after injection. Autopsy of this dog has demonstrated that the removal of the salivary glands had been complete. This work will be described in detail in another paper. The question of hypertrophy of the glands of the buccal mucosa after extirpation of the salivary glands has not yet been investigated.

CONCLUSIONS

Total extirpation of the salivary glands in dogs does not increase their average daily water intake. Therefore it appears improbable that the salivary glands play a major rôle in the thirst mechanism.

After total extirpation of the salivary glands in dogs the buccal mucosa remains in a healthy and remarkably moist condition.

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STUDIES OF CARDIAC OUTPUT IN NORMAL MEN

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The ethyl iodide method for measurement of cardiac output used in this laboratory (Starr and Gamble, 1928a) differs from that of Henderson and Haggard (1925) by the addition of a technique to determine the ethyl iodide content of mixed venous blood and the use of a different distribution coefficient for ethyl iodide in air and blood.

The purpose of this communication is to report experiments on the fundamental principles of the method which confirm those published previously, to answer certain criticisms, and to give the results of over one hundred determinations on normal young adult males.

These results show good agreement of duplicate determinations. They indicate an average level of cardiac output in the resting post-absorptive state which agrees closely with that found by a number of other methods. They disclose changes after meals, in the cold, on change of position and in excitement which are similar to those found by the use of the Krogh and Lindhard method and its modifications.

TECHNICAL ARRANGEMENTS AND PROCEDURES. *Alterations in arrangement of apparatus.* The arrangement of the alveolar circuit has been changed to save time in making duplicate determinations (see fig. 1). The valve and mouthpiece (Starr and Gamble, 1928a, fig. 4) has been altered so that a rubber Rosling valve has been substituted for the flap valve (fig. 2).

Technique of the determination of cardiac output. This has not been materially changed. As a rule the subject had inhaled ethyl iodide for 20 minutes before the first of the duplicate determinations, the second was made about 10 minutes later. Sampling tubes of 500 cc. capacity were employed. The silver nitrate method (Starr and Gamble, 1927) was used for all analyses.

Calculation and correction of results. The figure usually used for volume of respiration is that for the five minute period before rebreathing. The average distribution coefficient, 6.1, has been employed throughout.

The small correction for necessary delay in taking the rebreathed sample (R sample) has been discussed at length (Starr and Gamble, 1928a, pp.

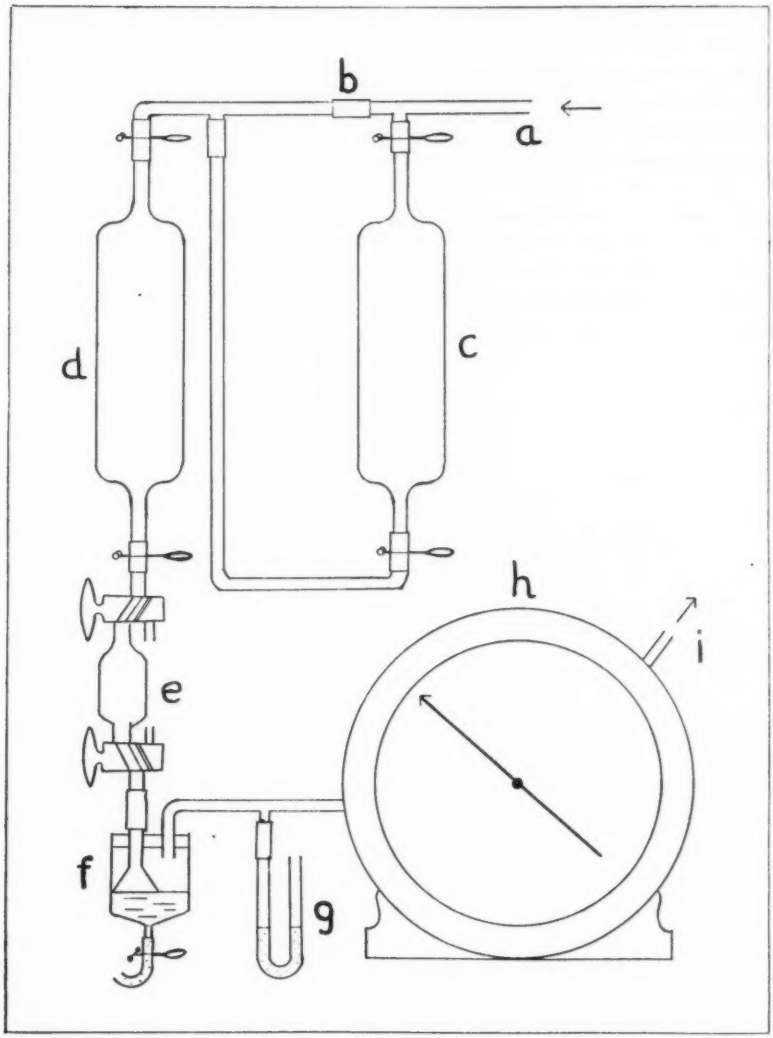

Fig. 1. Diagram of circuit for collection of alveolar ethyl iodide and carbon dioxide. *A*, connection from mouthpiece; *c* and *d*, ethyl iodide sampling tubes; *e*, Haldane sampling tube for CO_2, *f*, Mueller valve, leveling bulb not shown; *g*, water manometer; *h*, Bohn meter; *i*, connection to mouthpiece.

During the first of two duplicate determinations the tube at *b* is closed with a hemostat; during the second the tubing at *b* is open, the pinchcocks of sampling tube *c* are closed. At least 2½ liters should pass through the circuit before sample *e* is taken; *d* may be taken after an additional liter has passed through.

Alveolar CO_2 is estimated from the air in *e* trapped at the end of the experiment.

467–470). We have corrected our results as described before, employing curves constructed from the R values of similar experiments. If similar experiments are not available we have utilized curves made as follows: the R values of duplicate determinations are plotted against the duration of inhalation of ethyl iodide; a curve roughly parabolic in shape is drawn free-hand through these points to the origin as vertex.

When using subjects in the horizontal position, if the duplicate determinations are made 20 and 30 minutes after the beginning of inhalation of ethyl iodide, the R values are usually so nearly alike that the correction may be neglected. In the upright position the rate of increase of the R value is greater and the correction is of more importance.

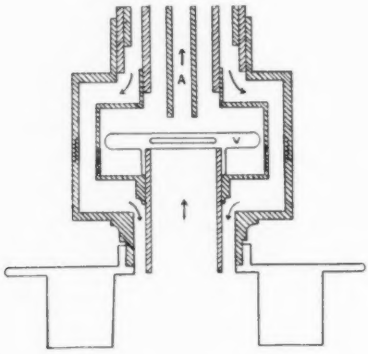

Fig. 2. Mouthpiece, expiratory valve, tubing for inspired and expired air. In order to permit the use of a rubber Rosling valve in place of the flap expiratory valve, the end of the combined mouthpiece and valves (Starr and Gamble, 1928b, fig. 4) has been altered to the form shown here. Only the part adjacent to the mouthpiece has been shown. The figure is in scale with the drawing illustrated in the previous communication, and the whole arrangement can be seen by superimposing this figure over the other, its upper edge being placed in line with the nearest side tubes.

During expiration the rubber valve, *V*, rises and closes the entrance of the alveolar circuit, *A*.

FURTHER EXPERIMENTS ON THE FOUNDATIONS OF THE METHOD. The majority of these experiments were performed to extend the use of the method to subjects in the upright position.

Experiments on the distribution coefficient. Because of the concentration of blood on prolonged standing reported by Thompson et al. (1928) distribution coefficients were determined upon the blood of C. S. drawn both after lying and standing for an hour as in an experiment to determine the effect of posture on cardiac output. The figure 6.2 was obtained in both cases. Blood oxygen capacities on H. after lying and standing gave no

evidence of blood concentration in the latter position under the conditions of our experiments.

Experiments on the equilibrium between ethyl iodide in alveolar air and arterial blood.[1] The demonstration that the ethyl iodide content of arterial blood may be estimated from that of alveolar air has been repeated with the subject standing. Blood was obtained from a vein on the back of the hand immersed in water at 45°C.; this technique again yielding blood of normal arterial oxygen saturation. A graphic record provided evidence that respiration did not change as blood was drawn. An unexpected difficulty was encountered in performing the experiment; four subjects experienced faintness and two collapsed (Starr and Collins, 1929). Five successful experiments were at length secured; the results are recorded

TABLE 1

Comparison of the ethyl iodide concentration of blood from a hand vein after immersion in water at 45° with that estimated from the alveolar air. Subject standing

DATE (1929)	SUBJECT	SIZE OF BLOOD SAMPLE ANALYZED	CONCENTRATION FOUND BY ANALYSIS	ESTIMATED FROM ALVEOLAR AIR AND AVERAGE NORMAL DISTRIBUTION COEFFICIENT = 6.1	ERROR
		cc.	*mgm. per 50 cc.*	*mgm. per 50 cc.*	*per cent*
January 19	C	46	0.58	0.59	+2
January 23	S	25	0.54	0.47	−13
February 1	C. S.	49	0.47	0.49	+4
February 12	H	43	0.39	0.36	−8
February 18	Gam.	36	0.49	0.56	+14
Averages			0.49	0.49	

Average error of samples analyzed = ± 0.03 mgm. or 8.2 per cent

in table 1. It will be seen that the largest errors occur when small amounts of blood were obtained for analysis and so the differences found lie within the analytical errors involved. The averages agree well.

In one other experiment the subject, unaccustomed to vein puncture, was excited, the respiration reaching 13 liters and the rate 35 per minute at the moment of puncture. The alveolar CO_2 was reduced to 4.44 per cent. The alveolar air contained 2.09 mgm. of ethyl iodide per liter, the blood 7.5 mgm. per liter. Obviously the content of the arterial blood could

[1] Our attention has been called to an error in transcription appearing in table 8 of a previous communication (Starr and Gamble, 1928a). In the experiment of May 12th the alveolar air contained 0.13 mgm. of ethyl iodide instead of 1.13 mgm. as there recorded.

not have been calculated from that of alveolar air in this experiment. In abnormal conditions of respiration automatic sampling cannot be relied upon to yield air in equilibrium with arterial blood.

Because of the equilibrium between CO_2 in arterial blood and samples of alveolar air secured by the Haldane-Priestley technique when the subject exhaled at the end of expiration (Bock and Field, 1924), we have made numerous comparisons between CO_2 content of alveolar air collected by this technique and that secured automatically. In 16 experiments on 8 subjects in the horizontal position the CO_2 of the automatic samples averaged 95.2 per cent of that of the Haldane-Priestley samples. In some subjects, S, H, and Ho, there was usually no significant difference between samples collected by the two methods. In 10 experiments on 4 standing subjects (C, S, H, and Ho) the average CO_2 content of the automatic samples was 101 per cent of that of the Haldane-Priestley samples; i.e., there was no significant difference.

TABLE 2

Ethyl iodide concentration in air rebreathed from a series of bags with the subject standing

SUBJECT	ETHYL IODIDE IN AIR REBREATHED 30 SECONDS	CHANGE AFTER 90 TO 95 SECONDS REBREATHING		SUBJECT	ETHYL IODIDE IN AIR REBREATHED 30 SECONDS	CHANGE AFTER 90 TO 95 SECONDS REBREATHING	
	mgm. per liter	*mgm. per liter*	*per cent*		*mgm. per liter*	*mgm. per liter*	*per cent*
C	1.17	−0.10	−9	G	0.92	−0.12	−13
G	1.14	−0.14	−12	S	0.88	−0.11	−12
C	1.14	−0.21	−18	C	0.86	−0.07	−8
C. S.	1.07	−0.15	−14	S	0.81	−0.12	−15
C	1.03	−0.17	−17	H	0.76	−0.06	−8

Experiments on the equilibrium of rebreathed air and venous blood and on the rate of destruction of ethyl iodide in the body. The experiments on men providing evidence that the ethyl iodide content of mixed venous blood can be estimated from that of rebreathed air (Starr and Gamble, 1928a, table 7) have been repeated. After inhaling the same concentration of ethyl iodide for fifteen minutes or longer the subjects rebreathed for 30 seconds into one bronze bag, then for 60 seconds into another. The results of ten experiments on subjects in the upright position are given in table 2. A comparison with similar experiments made with the subject reclining (Starr and Gamble, 1928a, table 7) discloses that the ethyl iodide content of rebreathed air diminished at a rate which was usually negligible when the initial concentration was small, but diminished faster when the concentration was larger. In the standing position the ethyl iodide in alveolar and rebreathed samples is usually higher than when the subject is horizontal, probably a result of increased respiration. The rate of fall

of ethyl iodide content of rebreathed air is accordingly greater in this position.

These results suggest two possibilities one or both of which may apply. First, the rate of destruction of ethyl iodide may increase as the amount in the body increases. We have never maintained, as Henderson and Mobitz (1930) imply, that the rate of destruction of ethyl iodide, demonstrated for one subject to be not greater than 13 per cent per minute (Starr and Gamble, 1928b), applied to all subjects and all experimental conditions. Second, the amount of blood rapidly returning to the heart when the subject stands may be larger than when the subject is horizontal, therefore the effect of destruction of ethyl iodide in the tissues on the venous tension would manifest itself sooner.

When the R value is high and the fall more rapid we have not as much confidence in our ability to estimate the ethyl iodide content of mixed venous blood. Nevertheless the error, if any, is probably small and usually confined to the standing position; its direction would make the recorded blood flows smaller than the actual when the subject stands. This matter has been discussed at length (Starr and Gamble, 1928a), but we have recently lost confidence in the belief there expressed that no blood returns to the lungs in 15 seconds (Starr and Collins, 1930).

DETERMINATIONS OF CARDIAC OUTPUT. *Consecutive determinations in the resting post-absorptive condition.* The subjects were healthy males between 22 and 36 years of age who came from the suburbs to the laboratory without breakfast and remained lying for at least an hour and a half before the first of the duplicate determinations. A sample of expired air, obtained from the mixing bottle after stirring, was slowly collected during the second determination of cardiac output and used to calculate the metabolism, employing the respiratory quotient determined. This technique permits almost simultaneous determinations of blood flow and metabolism. The results are recorded in table 3. The normal basal metabolisms were calculated from the tables of DuBois.

Only on subject F did duplicate determinations of cardiac output fail to yield results agreeing within the error of determination. F was not accustomed to respiratory procedures, and in such cases it is not unusual to find the first determination higher than the second, doubtless due to initial excitement; Co, also a novice, was obviously excited as his metabolism and respiration testify. Subjects E and R were dozing. Omitting the first determination on F the average deviation of each subject's cardiac output from the mean of the series is 16 per cent; that of the cardiac outputs per square meter is 13 per cent. The arterio-venous oxygen differences, calculated by dividing oxygen consumption by cardiac output, have an average deviation of 11 per cent. The ratios, cardiac output per square meter divided by oxygen consumption show an average deviation of 8

TABLE 3

Cardiac output of normal men

1. IN THE RESTING POST ABSORPTIVE STATE				2. EFFECT OF CHANGE OF TEMPERATURE				3. EFFECT OF CHANGE OF POSTURE			4. EFFECT OF EXCITEMENT, FOOD, SLEEP, ETC.		
Subject	Cardiac output		Metabolism	Subject	Cardiac output	Oxygen consumption	Remarks	Subject	Cardiac output	Position lying or standing	Subject	Cardiac output	Remarks
	liters per minute	*liters per square meter per minute*	*per cent of normal basal*		*liters per minute*	*cc. per minute*			*liters per minute*			*liters per minute*	
F	4.7			G	3.3		Warm	C	6.0	L	S	5.4	Vein punctured but not excited
	3.8	2.1			4.9		Cool		6.5				
					4.1		Cold		5.9	S			
E	3.1				3.6		Warm		5.9		C	6.1	Vein punctured—a little excited
	3.1	1.56	−20										
				S	5.6		Warm	C	4.6	L			
C. S.	4.3				7.0		Cold		6.0		H	10.2	Fears he may faint
	4.2	2.16	−14		4.3		Colder		4.9	S		9.5	Vein punctured, still excited
									5.2				
R	3.5			C		289	Warm						
	3.2	1.72	−17		5.1	312	Cold	S	6.6	L	S. S.	12.5	Anticipates fainting
					5.75	288	Colder		6.5			7.5	Vein punctured, confidence returning
Co.	4.0				4.0	261	Warm, dozing		6.6	S			
	4.1	2.24	+17										
				C	8.9	386	Warm, excited	S	5.8	L	C. S.	6.9	Anticipates fainting
C	4.4				7.9	448	Cold		5.8				
	4.1	2.08	−13		9.6	389	Colder		5.5	S	Gam.	4.5	Vein punctured, not excited
					9.3	353	Warm, not relaxed		5.8				
Wr.	5.2		−4.5										
	5.5	2.76	−2					S	5.1	S	C	5.9	2 hours after lunch
				S	6.5	254	Warm		5.8			6.2	Duplicate

G	3.6				5.3	367	Cold		4.6	L		5.6	1½ hours later
	3.6	2.01	+2		11.4	395	Very cold		4.4			5.5	Duplicate
					6.2	315	Warm, not relaxed	C. S.	7.0	L	S	4.8	Before lunch
K	2.65								5.9			5.1	Duplicate
	2.8	1.69	−14						5.5	S		7.6	1 hour after lunch
				S	6.4	295	Cool		5.9			6.4	Half hour later
S	4.9				6.0	—	Warmer						
	5.3	2.5	−7		5.7	288	Too warm						
Averages omitting first determination on F,					5.4	272	Cooler	H	3.6	S	G	4.3	Sleepy
									3.3			3.6	Asleep
									4.5	L			
	3.97	2.08							4.5		T	4.3	After 15 minutes rest
												3.9	10 minutes later
								H	4.6	S		3.7	30 minutes later
									4.8			3.6	25 minutes later
									5.7	L			
									6.0				

The figures for cardiac output given in divisions 1 and 3 are the results of duplicate determinations made when conditions were constant.

In divisions 2 and 4 the physiological condition changed between each determination except where duplicates are indicated.

per cent; if the experiment on Wr. who had cardiac disease in childhood is omitted, this deviation becomes only 6 per cent. This is the most constant relationship shown by our subjects.

The effect of the duration of the preliminary rest period. The result of an experiment of this type is recorded in table 3, division 4. Another has been reported previously (Starr and Gamble, 1929a, table 9). These and other experiments showing the same result have caused us to prolonged the preliminary rest period beyond that required to secure basal metabolism. The subjects must travel over one-half hour before reaching the laboratory and we employ a rest period of about one and a half hours before a determination of basal blood flow. The success of this procedure in most subjects is shown by the duplicate determinations recorded in table 3.

Effect of food. Two experiments showing the rise of cardiac output after meals and its subsequent fall are recorded in table 3. A comparison of initial determinations of experiments made before and after meals shows the same thing.

Effect of sleep. In one fortunate experiment (table 3) the subject, sleepy during the first determination, slept soundly during the second. His cardiac output fell to his basal level. We believe that this experiment is unique. Two subjects who were dozing also had basal cardiac outputs.

Effect of change of temperature. The subjects lay down without clothing but covered with blankets. To cause chilling the blankets were removed and the breeze from an electric fan allowed to play on the subject. In one experiment, the subject was warmed by piling on extra blankets. Temperatures were taken by mouth, the thermometer being left in until constant readings were obtained. Body temperature diminished 0.6 to 1.6°F. during chilling.

The striking feature of the results (table 3) is the variability of the cardiac output and metabolism during the chilling. It appears that a slight degree of chilling may cause a rise in cardiac output if this was initially low, a fall if it was formerly high. Severe chilling was accompanied by a rise of cardiac output in three cases, a fall twice. The fall in cardiac output and metabolism after the subject had become warm again was observed in every experiment. At other times, the metabolism frequently varied in a different direction from the cardiac output.

The one experiment in which the subject was made uncomfortably warm shows a steadily diminishing cardiac output and metabolism which might properly be attributed to the increasing degree of relaxation obtaining during the experiment.

Effect of change of posture. This, the most debated subject in this field, is difficult to investigate because of the profound change in respiration, dead space, alveolar CO_2, and the residual air which occurs on change of position. The typical difference between the ethyl iodide content of ex-

pired, alveolar, and rebreathed samples in the lying and standing positions is illustrated in table 4; the magnitude of this difference makes the essential similarity of the calculated cardiac outputs the more impressive.

The majority of the determinations on subjects C. and S. (table 3, division 3) as well as that on C. S. show either no change on standing or a fall so small that the significance is doubtful. Another subject, B., gave a similar result but his respiratory rate was so unusually slow that we have not full confidence in the determination. In the last experiment on S. the higher results standing should be attributed to excitement. On the other hand, subject H. showed constantly a smaller cardiac output standing than lying, a result also obtained in an experiment not included in table 3.

Effect of excitement and alarm. After a number of persons had fainted during vein puncture in the standing position (see page 232) this experiment alarmed the subjects. The cardiac output has been determined five times

TABLE 4

Posture experiment on subject S, January 7, 1929

ETHYL IODIDE				RESPIRATION	PULSE	CARDIAC OUTPUT	OXYGEN CONSUMPTION	ALVEOLAR CO_2	POSITION
Inspired	Expired	Alveolar	Rebreathed						
mgm. per liter	*mgm. per liter*	*mgm. per liter*	*mgm. per liter*	*liters per minute*	*per minute*	*liters per minute*	*cc. per minute*	*per cent*	
5.64	2.51	1.09	0.55	5.6	80	5.8			Lying
5.68	2.39	1.10	0.53	5.6	74	5.8	271	5.3	Lying
5.82	3.16	1.97	1.32	7.45	92	5.5			Standing
5.73	3.29	2.02	1.46	7.45	90	5.8	316	4.9	Standing

Temperature correction = 91.6 per cent.

in three subjects who underwent the experiment in a state of real excitement and apprehension. Two types of control experiments are available: determinations on the same subjects standing with mind at ease (table 3), and estimations on other subjects who, accustomed to vein puncture and ignorant of or indifferent to the hazard of fainting, went through the same experiment without excitement. The results have been placed in table 3, division 4.

Subjects C. and S. completed the experiment before anyone had fainted. S. was accustomed to vein puncture and neither felt nor exhibited excitement; C., less experienced, showed slightly higher cardiac output and greater respiration than in control experiments. He felt slightly excited.

Subjects H. and C. S. had fainted during previous experiments but consented to try again. The results recorded were obtained during the second attempt while the subjects were conscious of very real alarm. H. exhibited a great increase in cardiac output and respiration before the vein was punc-

tured. C. S., a less marked increase. Neither fainted when the needle was inserted. S. S. who volunteered after several others had fainted began the experiment in much alarm but, when he developed no tendency to faint, regained confidence. Gam., though aware of the hazard, was not excited.

The results show the large increase in cardiac output which may be caused by emotion. The most complete data were obtained on S. S. During marked excitement when his cardiac output was 17.5 liters per minute, the pulse rate was 100; respiration was 13.5 liters per minute, the rate 12; blood pressure was 122/77, and O_2 consumption 470 cc. per minute. Later, as excitement waned, his cardiac output fell to 7.5 liters per minute and his pulse to 84, but his respiratory rate rose to 17 per minute and the respiratory minute volume, blood pressure and metabolism remained at their former levels. The normal basal oxygen consumption of this subject was calculated to be 273 cc. per minute.

No results obtained while a subject felt faint have been included in table 3. These will be considered elsewhere and have been reported in abstract (Starr and Collins, 1929).

Discussion. *Calculation of the dead space as a check on the reliability of results.* Mobitz (1926), and Henderson and Mobitz (1930) suggest that if the dead spaces found by carbon dioxide and ethyl iodide do not agree closely a technical error is present and the experiment should be discarded. We do not follow this suggestion because: first, the size of the dead space (thus determined) is not usually the same for different gases (Haldane, 1915; Henderson, Chillingworth and Whitney, 1915); second, no evidence that the dead space determined by C_2H_5I constantly agrees with that estimated by CO_2 has been presented; lastly, our data show such agreement in only about 50 per cent of instances when duplicates give no indication of technical error.

Reasoning from the same point of view, Henderson and Mobitz (1930) suspect analytical errors in one of our experiments (Starr and Gamble, 1928a) because the dead space calculated from ethyl iodide is of different size in each of three consecutive determinations. But somewhat larger differences, found in similar consecutive estimations on one subject by Henderson, Chillingworth and Whitney (1915), were attributed to variation in size of the dead space. This explanation seems applicable to our results also.

The same authors have criticised two of our animal experiments (Starr and Gamble, 1928b) because the ratio of respiration to circulation would have to be very large to account for the results. The amounts of ethyl iodide recovered from the blood having been small, the errors inherent in the method of analysis (Starr and Gamble, 1928a) and the irregularity of respiration during the experiment will explain the findings.

The accuracy of results and the significance of differences. All investigations involving cardiac output are handicapped by the impossibility of determining the size of the absolute error of the method employed; therefore, this error should be regarded as large.

In results on the same individual under similar conditions the deviation of duplicate determinations from each other permits estimation of the size of significant differences. We find the average difference between duplicates in twenty-eight consecutive experiments on twelve subjects to be 0.33 liter per minute or 6.4 per cent of their mean; in only four is this difference greater than 10 per cent.

When the cardiac output is compared in conditions in which the respiration or circulation has changed (e.g., change of posture; rest and exercise; health and disease) the deviation of results under constant conditions is not a proper criterion of the significance of the differences found. We have decided to accept changes of 25 per cent as significant in such experiments, although we believe that our method is more accurate than this figure implies.

Comparison of our results with those of other methods. The cardiac outputs of our subjects in the resting post absorptive state agree well with that found by many other methods, viz.: Krogh and Lindhard (Liljestrand and Stenstrom, 1925); Marshall and Grollman (1929); Grollman, (1929a); Redfield, Bock, and Meakins, (Hayasaka, 1927); Eppinger, Papp, and Schwarz, (1924); Burwell and Robinson (1924); and Lauter (1930a and b). The average of our results per square meter is slightly lower than that obtained by the other authors. The average metabolism of our subjects is farther below the calculated basal level than that found by Liljestrand and Stenstrom (1925) and Grollman (1929a); the data available do not permit a similar comparison with the other series. Therefore we attribute our lower results to the fact that our method, neither requiring the attention of the subject, nor employing cardiac, nor arterial puncture, permits more complete relaxation than can be obtained when the other methods are employed. Our long preliminary rest period may be a factor also.

Two widely used methods (Henderson and Haggard, 1925; and Field, Bock, Gildea, and Lathrop 1924) give results which indicate larger cardiac outputs in reclining subjects than are found by the methods mentioned before. We have already stated our reasons for believing the former method to give erroneous results (Starr and Gamble, 1927, 1928a and b). Lehman (1928, 1929) has come to a similar conclusion. Lauter (1930), who has compared the Henderson and Haggard method with the cardiac puncture method in man finds the results of the former to be about 30 per cent too high. The average result of our determinations performed according to the original Henderson and Haggard technique on subjects S. and G. in the basal condition (Starr and Gamble, 1927, page 509–510) is 36 per

cent higher than the average result of comparable experiments performed more recently by our method. When the cardiac output is calculated according to Henderson and Haggard from the data we obtained after modifying the method, the majority of results show larger differences from the figures which we regard as correct. These differences diminish as the duration of inhalation of ethyl iodide increased. As this duration has not been uniform in our experiments or in those of most other authors, we do not believe that the results obtained by the Henderson and Haggard method can be corrected by any single factor.

The reason for the divergence of our results from those obtained by the method of Field, Bock, et al. is not apparent to us.

Our results on the effect of food on cardiac output are essentially similar to those of other authors (Collett and Liljestrand, 1924; Jarish and Liljestrand, 1927; Grollman, 1929b).

Chilling produced irregular results on our subjects. Collett and Liljestrand (1924) report increased cardiac output on chilling. Marshall (1930) cites results showing a decrease. Lindhard (1915) and Barcroft and Marshall (1923) obtained more irregular results. Differences in personal reaction to cold are certainly to be expected.

We obtained much larger increases of cardiac output during excitement and alarm than were found by authors using less violent psychic stimulation. (Collett and Liljestrand, 1924; Grollman, 1929c).

Our results on the effect of posture resemble those of Grollman (1925). Certain of Turner's subjects (1927) also showed changes identical with our results. But the average decrease of cardiac output on standing found both by Turner (1927) and by Field, Bock, et al. (1924) was much larger than in our subjects. The difference is due to the higher results obtained by the Field-Bock method in the reclining position. The original ethyl iodide method also indicated a decreased cardiac output when the subject stood (Henderson and Haggard, 1925; Rosen and White, 1926) and by using the same method of calculation, this result can be obtained from our data. As soon as proper allowance is made for ethyl iodide returning to the lungs the difference between the calculated cardiac output lying and standing disappears in most of our subjects.

Relationship of cardiac output to metabolism. In different subjects in the basal condition and in the same subject on successive days an imperfect direct relationship between cardiac output and metabolism appears. Change of cardiac output and metabolism in the same direction occurred after food, during excitement and after chilling. This direct relationship was first pointed out by Lindhard (1915). But on change of position and during chilling the cardiac output and metabolism diverged in some of our subjects.

Comparison of cardiac output with clinical type. Subject C. has a highly

reactive type of circulation; he blushes easily and repeated estimations of systolic blood pressure vary widely, figures anywhere between 170 and 120 mm. of Hg being secured. The other subjects have stable circulations and consistent normal blood pressures. We have detected no significant difference between C.'s cardiac output and that of our other subjects in any of the conditions studied.

SUMMARY

Experiments on the fundamental assumptions of the improved ethyl iodide method for the determination of cardiac output were repeated and yielded results which increased our confidence in the method.

This method yielded duplicate determinations whose average difference was 6.4 per cent of their mean. The results demonstrate that the cardiac output of resting normal adult males is essentially constant. The average level in ten normal young adult males in the post absorptive condition, after a prolonged rest period in the horizontal position was 3.97 liters per minute or 2.08 liters per square meter of body surface. These results are quite similar to those of Grollman.

The experiments indicated that food and excitement increase cardiac output. Cold caused changes in both directions; becoming warm after having been chilled was always accompanied by a decrease of cardiac output. The change from the lying to the standing position caused no significant alteration in four subjects; in one subject the cardiac output standing was constantly lower than that lying. These results are in satisfactory agreement with those of many other methods.

A subject having a highly reactive type of circulation with inconstant blood pressure showed no difference in general level of cardiac output, nor in response to food, cold, position, and excitement than the other more stable subjects.

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AN EXPERIMENTALLY PRODUCED PREMATURE SYSTOLIC ARHYTHMIA (PULSUS BIGEMINUS) IN RABBITS

III. Pathway of the Arhythmia Impulse

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It would appear from the two previous papers of this series that a bigeminal pulse elicited from benzol insufflation and other means was brought about in some way by a direct nerve impulse or impulses to the heart, which was shown by a process of elimination should descend the spinal cord and reach the left ventricle by way of the sympathetics. The purpose of this paper is 1, to make certain that this impulse traverses the spinal cord; 2, to determine the region of the spinal cord utilized by the impulse; 3, to trace the impulse peripherally to the heart.

Effect of severing the vagi, depressors and removing the inferior cervical sympahetic ganglia. It was attempted in these experiments to exclude every known pathway from the brain to the heart except the spinal cord route. The above mentioned nerves and ganglia were transected or removed in 6 rabbits under veronal sodium and light ether. In 4 of these animals the carotid arteries were also denervated from the subclavians up to and including the carotid sinuses and both phrenics were cut. Cannulae were inserted into the trachea and pharynx and the arhythmia tests were conducted after the manner described in the first paper of this series.

One period of benzol insufflation was sufficient to produce a bigeminal pulse in 4 of these rabbits, while 2 required an additional period. Figure 1 A shows a bigeminal pulse obtained from the first insufflation of benzol preceded by an unintentional mechanical stimulation of the nasal septum by the nose piece of the insufflation bottle in one of the rabbits which had its carotid sinuses denervated and phrenics cut in addition to sectioning the vagi, depressors and removing the inferior cervical sympathetic ganglia. The tracing discloses a 53 second interval of bigeminal pulse (a 40 second interval being omitted from the figure). It is of especial interest to note that the pulse rate shows no apparent change in this or any of these records, while the pulse is generally strengthened as in normal animals. As observed previously for vagotomized rabbits, two intervals of bigeminal pulse

were often evoked from one benzol insufflation in some of these animals, after which the normal rhythm was always maintained until further stimulation.

Effect of transecting the spinal cord in the lower cervical region. In these experiments the spinal cord was sectioned 2 days to 3 weeks before the arhythmia tests were made. Only 4 rabbits were used in these experiments, but this number is sufficient in view of the mass of corroborative

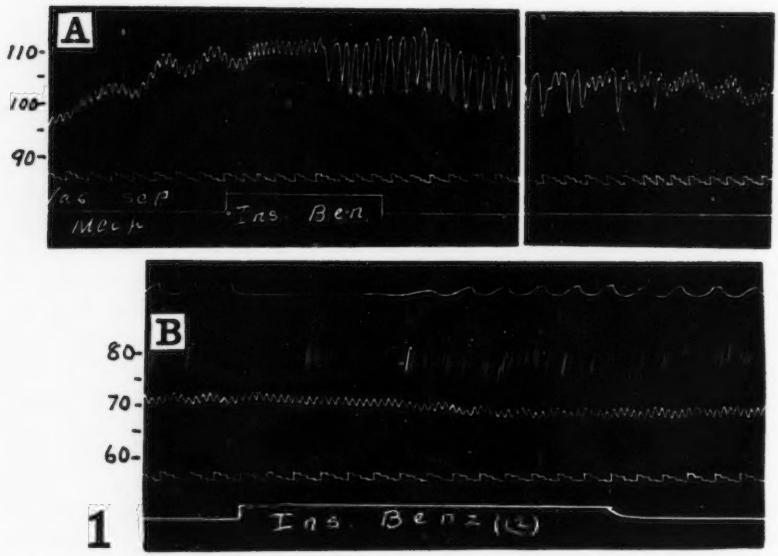

Fig. 1A. Carotid tracing during mechanical stimulation of the nasal septum and benzol insufflation from rabbit 659, which had vagi, depressors, cervical sympathetics and phrenics sectioned, inferior cervical sympathetic ganglia removed and carotid sinuses denervated. Omitted portion represents a 40-second interval of the arhythmia, time in seconds.

B. Thoracic respiratory and carotid tracings at the time of benzol insufflation from rabbit 687, which had spinal cord transected at 6 C.

evidence to be presented later in connection with sectioning certain thoracic spinal roots, the removal of the stellate ganglia and numerous partial transections of the spinal cord in the lower cervical region. The regular procedure was adopted in making these tests. All animals were in good condition with blood pressures only a little below normal and all reflexes which are not blocked by sectioning the cord were present.

The rabbit selected to represent the other three had its spinal cord sectioned 3 weeks before the arhythmia tests were made. This animal re-

ceived a three-fourth anesthesia dose of veronal sodium and light ether during the second operation which included the removal of the inferior cervical sympathetic ganglia. This animal, a small rabbit, had a blood pressure of 70 mm., yet no bigeminal pulse resulted from 16 periods of benzol insufflation. It then became necessary to discontinue this method of stimulation because it suddenly failed to produce the ordinary respiratory inhibition. The left vagus was sectioned and some 60 faradic stimulations of its central end did not elicit an arhythmia. The twelfth insufflation record (fig. 1 B) is representative not only of the other tracings from this animal but of all the tracings from the other aimals. The respiratory graph above discloses an arrest of respiration and the carotid tracing below reveals a slight drop in blood pressure and no pulse changes.

Effect of transecting the spinal cord at various levels from the 3–9 thoracic vertebrae. The spinal cord was sectioned at the level of the 3 T in three rabbits, at the 4 T in two rabbits and at the 6, 7, and 8 T in one rabbit each. These operations were completed 3 days to 2 weeks before any arhythmia tests were made. All animals were in condition to give a bigeminal pulse when tested.

Of the three rabbits in which the spinal cord was transected at the 3 T, rabbit 680 responded with a bigeminal pulse of the second type from the fourth insufflation of benzol. The carotid tracing shows the arhythmia to be preceded by a very small rise in blood pressure, some slowing and strengthening of the pulse and an arrest of respiration. Successive insufflations were followed by similar arhythmias. The other two rabbits and the two in which the spinal cord was transected at the 4 T did not yield an arhythmia from twenty or more benzol insufflations. A bigeminal arhythmia followed the seventh benzol insufflation in the rabbit in which the spinal cord was sectioned at the 6 T and with the first insufflation in the rabbit in which the spinal cord was cut at the 8 T.

One bigeminal arhythmia tracing from the rabbit in which the spinal cord was transected at the 7 T appears in figure 1 C in the second paper of this series, being one of the tracings introduced to show that a bigeminal pulse brought on from insufflation may take place without being preceded by a rise in blood pressure. The spinal cord of this animal was sectioned one week before the arhythmia tests were made, and the rabbit, a small animal, had a blood pressure of 80 to 90 mm., yet it required 20 insufflations of benzol to bring on the first bigeminal arhythmia. This arhythmia, however, was readily evoked with each succeeding benzol insufflation.

After comparing the results of these experiments with those of the following stellate ganglia and spinal root experiments, the possibility suggests itself that the non-appearance of the bigeminal pulse in many of the above experiments where the spinal cord had been transected in the region between the 3 T and 5 T might be attributed to a clot in the gray matter of

the spinal cord or other effects of trauma which extend cephalad to involve the efferent cells for the heart.

Significance of the stellate (first thoracic sympathetic) ganglia. These ganglia are much more difficult to remove in rabbits than they are in dogs and cats, especially the one on the right side, which consists of an elongated mass of cells extending as far cephalad as the subclavian artery.

A considerable number of preliminary tests were made to ascertain the effect of trauma and shock of opening the thorax and cutting the pleura on blocking the bigeminal pulse following benzol insufflations,—in other words to determine the validity of acute experiments. It has been demonstrated that many bigeminal arhythmias have been obtained easily from benzol insufflation and the other means used in several rabbits: *a*, after the thorax had been opened on both sides (see fig. 1 A, second paper of this series); *b*, after opening the thorax and cutting both sympathetic cords; *c*, after opening the thorax, removing one stellate and sectioning the opposite sympathetic cord directly below the stellate. In *b* and *c* the thorax was generally closed and normal respiration maintained, though some arhythmias were obtained during artificial respiration. There can be no question from these and many other tests but that this "arhythmia impulse" is one of the last impulses to disappear in any animal in which the nervous path to the heart is intact.

The procedure used in removing the stellates is as follows: After the usual anesthesia (veronal sodium and ether) and insertion of the tracheal and pharyngeal cannulae, the left M. pectoralis major and the M. pectoralis minor were separated, a slit was made between the second and third ribs and an opening of considerable size was obtained by separating the two ribs by a small retractor commonly used in mastoid surgery. All branches of the stellate were cut and the ganglion removed. The ribs were closed by two ligatures, the last one being tied when the lungs were fully expanded by the pulmotor, after which the pectoralis muscles and skin were sutured. The right stellate ganglion was removed after the same manner as the left, except that it was generally necessary to destroy the cephalic end of the ganglion by cauterization of the M. longus colli. It was advisable to use artificial respiration while working in the right side of the thorax. Autopsies were made on all animals and if there was any doubt as to the presence of a piece of ganglion on the right side, that portion of the sympathetic trunk was sectioned and studied microscopically. All of the nine animals reported were in good condition throughout the tests.

Figure 2 A, the sixteenth benzol insufflation from rabbit 733, is not only representative of nineteen others from this animal, but of all the tracings from each of the eight animals in which the stellates were removed and some 20 insufflation tests were made. It is apparent that this graph shows no bigeminal pulse. It discloses some rise in blood pressure, an arrested

respiration, a slightly strengthened pulse and little or no change in pulse rate.

In addition to the nine negative animals in which both stellates were completely removed there were many more unreported negative animals whose autopsies showed the left stellates completely extirpated and only remnants of the cephalic end of the right ganglia. There is a strong probability that the right stellates were not functioning in these animals, but inasmuch as the interpretation of these tests depends on negative evidence these animals must be discarded.

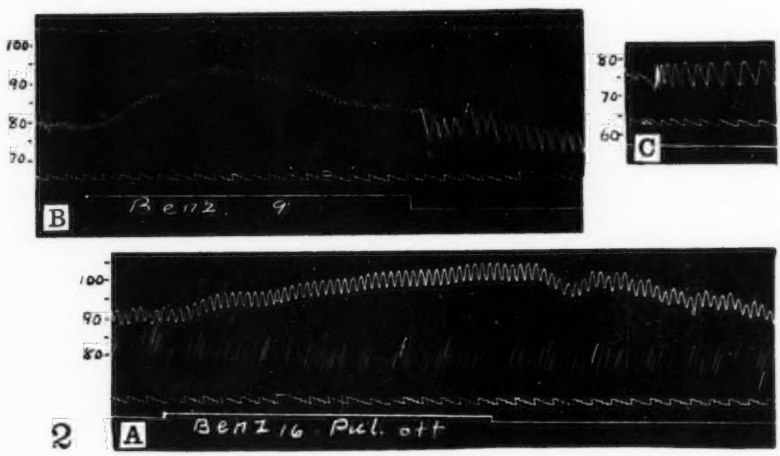

Fig. 2 A. Carotid tracing during benzol insufflation from rabbit 733, which had both stellates removed, no arhythmia.

B. Thoracic respiratory and carotid tracings during benzol insufflation from rabbit 708, which had left stellate removed and right sympathetic cord cut behind the stellate. Observe bigeminal arhythmia in carotid tracing.

C. Carotid tracing showing bigeminal arhythmia from benzol insufflation, rabbit 734, which had right stellate removed and left sympathetic cord cut below the stellate.

Effect of removing one stellate ganglion. Following a suggestion of Doctor Foster's that one sympathetic cord might be of greater significance than the other for the conduction of this "arhythmia impulse," a number of arhythmia tests were carried out in several rabbits in which one stellate was extirpated. In some of these animals the opposite side of the thorax was opened and then closed without injuring the sympathetics, while in others the opposite sympathetic cord was cut directly behind the stellate. In general when the right stellate was removed the cephalic end of the symphathetic trunk was cauterized to make certain that the entire ganglion was destroyed. This was checked by autopsy.

In two rabbits where the left stellate was extirpated and the right thoracic cavity was opened and then closed with no interference to the sympathetic chain, a bigeminal pulse followed the fourth benzol insufflation in one rabbit and the tenth in a second rabbit. In two rabbits in which the left stellate was removed and the right sympathetic trunk was transected immediately below the stellate, it required seven and ten insufflations of benzol to elicit the first bigeminal arhythmia. A carotid tracing of a bigeminal pulse (type 1 variety) from one of the last mentioned animals (fig. 2 B) shows the arhythmia late in appearing.

If any deduction can be made from the arhythmia tests from animals in which the left stellate has been removed, it is, that the insufflation arhythmia' may have been delayed somewhat by the removal of the ganglion.

Two rabbits which came through the operation of removal of the right stellate ganglion with blood pressures of 110 mm. and 105 mm., failed to give a bigeminal arhythmia from 28 and 21 benzol insufflations. Two other rabbits which came through the operation with similar blood pressures yielded bigeminal arhythmias from the tenth and eleventh insufflations; while the fifth rabbit which had the left sympathetic trunk transected behind the stellate in addition to having the right stellate removed gave the arhythmia from the second insufflation. A small portion of this arhythmia appears in figure 2 C.

The above rather limited series of rabbits in which the right stellate ganglion was removed shows two negative arhythmia animals and three positive arhythmia animals. If the same ratio of negative to positive animals is sustained in a much larger series of animals, it could be assumed that the right stellate included the important arhythmia pathway for some rabbits.

Effect of cutting the ventral roots of certain spinal nerves intraspinally. In these experiments the dorsal and ventral spinal roots were sectioned 2 days before the arhythmia tests were made in order that the animals might recover from spinal shock. After a test was completed the lesion portion of the vertebral column was hardened in formalin and the spinal cord was carefully dissected to determine which spinal roots had been transected.

Two rabbits served for controls. In one, the spinal cord was exposed in the region of the lower cervical and the upper thoracic vertebrae and in the other, several spinal roots were sectioned in this region, but only the roots on one side in any one segment. Both rabbits yielded a bigeminal arhythmia from the first benzol insufflation. After six arhythmias were obtained from the last mentioned rabbit, the vagi, depressors and cervical sympathetics were cut at the level of the larynx and both the first and second benzol insufflations were followed by bigeminal arhythmias.

The last cervical and the first and second thoracic spinal roots were transected in two rabbits and the first three pairs of thoracic roots were sectioned in a third rabbit. All three rabbits were in good condition 2 days

after the operations, when 20 insufflations given each animal failed to produce an arhythmia. The carotid tracings exhibit the usual rise in blood pressure and arrest of respiration, but show little or no pulse changes.

The above rather limited series of spinal root experiments indicates that the "arhythmia impulse" ordinarily leaves the spinal cord by way of upper thoracic roots.

Area in the spinal cord which conducts this arhythmia impulse. The procedure used in this set of experiments consisted of making a certain partial transection of the spinal cord in the region of the 6th cervical vertebra with a view to finding an area which blocks the bigeminal arhythmia induced from insufflations and other means. This was accomplished 24 to 48 hours before the arhythmia tests were made. Median transections were made with chisels of varying width and in this mode of sectioning the cord it was generally advantageous to first strip off the dura from the field of operation. Lateral transections were made with a narrow straight-backed scalpel and for the transections designed to cut all of the fibers of the lateral column without injuring the dorsal and ventral columns it was advisable to cut the lateral column and dura with one stroke of the knife. All animals were in good condition throughout the arhythmia tests with blood pressure but little below normal and all reflexes which could pass up and down the cord did so. The arhythmia tests were conducted after the same manner as the previous experiments.

Upon completing an arhythmia test the lesion part of the spinal cord inclosed in the vertebral column was fixed and hardened for an hour or more in an alcohol-formalin-acetic acid mixture, after which the cord was removed and further fixed. Serial sections were prepared in a transverse plane and stained with hematoxylin and orange G. In some series one section included all of the lesion, but in most series the lesion involved different parts of several sections, so that it was necessary to make a composite drawing of these sections with the aid of a projector to obtain the full extent of the lesion in cross section. A number of such drawings appear in figure 3 and the lesion is represented by stippling.

An attempt was made in 4 rabbits to make a median transverse section of the spinal cord with a 2.5 mm. chisel which would sever the dorsal and ventral columns. After completing this incision in two animals, an attempt was made to cut the fibers below the ventral horns, the *latero-ventral columns*,[1] by means of a lateral movement of the edge of the chisel.

Microscopical sections demonstrate that the lateral and ventral columns

[1] In order to designate two parts of the ventral and lateral columns of the spinal cord it seems advisable to make use of two terms, namely, "latero-ventral column" and "ventro-lateral column"; the former to include the most lateral portion of the ventral column situated beneath the ventral horn and the latter, the most ventral portion of the lateral column situated laterally to the ventral horn.

were sectioned in all 4 rabbits and all yielded a bigeminal pulse readily from benzol insufflations. A reconstruction of the lesion in one of the rabbits in which an attempt was made to section the "latero-ventral col-

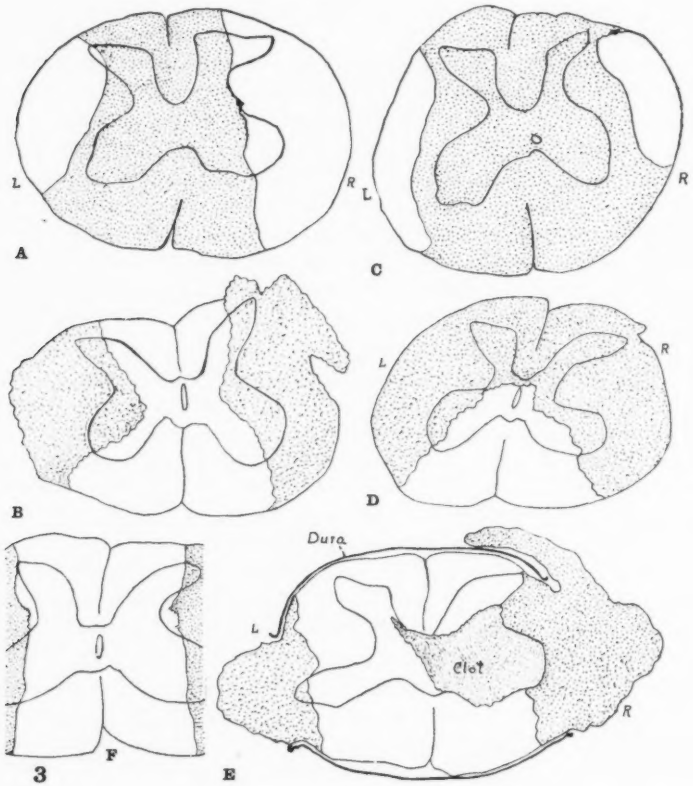

Fig. 3. Dark area of each transverse section indicates full extent of the spinal cord lesion. In some sections the illustration is from one section, but in most instances it is compiled from several sections.

A. Median transection involving the dorsal and ventral columns at 6–7 C; bigeminal pulse not blocked.

B. Lateral columns completely transected at 6–7 C; arhythmia blocked.

C. Dorsal and ventral columns and median half of the lateral columns transected; bigeminal pulse blocked.

D. All fibers but ventral column fibers transected at 6–7 C; bigeminal pulse blocked.

E. Lateral columns transected on right side and outer half of left lateral column sectioned at 6 C; bigeminal pulse not blocked.

F. Portion of a transverse section in which the lateral columns were sectioned except a small median area at 6 C; bigeminal pulse blocked.

umns" (fig. 3 A) shows the dorsal and ventral columns, the left "latero-ventral column" and a large part of the right "latero-ventral column" completely severed. During the interval of no stimulation this animal's blood pressure ranged from 105 to 90 mm. A bigeminal arhythmia of the type 2 variety was obtained from the second benzol insufflation. It appeared shortly after a 22 mm. rise in blood pressure, preceded by a distinct slowing and strengthening of the pulse and an arrest of respiration. A reconstruction of the spinal cord lesion of the other rabbit in which an attempt was made to section the "latero-ventral columns" in addition to the dorsal and ventral columns is very similar to figure 3 A and the first bigeminal arhythmia followed the eleventh benzol insufflation.

The lesion in figure 3 B from rabbit 764 at the level of the 6–7 C is representative of five others in which the lateral columns, including the "ventro-lateral columns" were completely transected. This lateral transection of the spinal cord absolutely blocked a bigeminal arhythmia from insufflations in all animals. In rabbit 764 where blood pressure ranged from 85 to 65 mm. during the period of no stimulation, no bigeminal pulse was elicited from 20 benzol insufflations and 4 faradic stimulations of the central end of the vagus. Carotid tracings during insufflations generally show a slight or no rise in blood pressure, little or no change in the pulse and an arrested respiration.

Figure 3 F shows the central portions of two lateral transections of the spinal cord in rabbit 774, which completely severed both lateral columns except for small central strips between the dorsal and ventral horns. These areas while appearing normal histologically may have contained but few functioning fibers at the time of the arhythmia tests. The lesions were made at the 6 C, 24 hours before the tests and blood pressure ranged from 80 to 60 mm. during the non-stimulation interval of the tests. As a result of 20 benzol insufflations there were no arhythmia or blood pressure changes, but respiration was arrested.

In 2 rabbits median transverse sections were made through the spinal cord at the 6–7 C with a chisel 3.5 mm. wide (1.5 mm. wider than the one used in the first of these cord experiments). Microscopical reconstructions reveal the two lesions to be identical. One of the reconstructions (fig. 3 C) shows the dorsal and ventral columns and the median half of the lateral columns completely severed. In addition to the areas included in the first spinal cord experiment, this lesion takes in all of the lateral ground bundle fibers next to the gray, which includes the lateral reticulo-spinal (bulbo-spinal) tracts. This animal went through the arhythmia test with blood pressure ranging from 95 to 65 mm. and 20 insufflations of benzol failed to evoke a bigeminal arhythmia in this or the other rabbit. All records disclose an arrest of respiration, a slight slow rise in blood pressure and little or no change in the pulse.

Transections similar to 3 D were made in 3 rabbits in the lower cervical spinal cord. In each instance the dorsal and lateral columns were completely severed, leaving the ventral columns for the most part uninjured. During the arhythmia tests blood pressure maintained a level of 70 mm. or higher. In each rabbit 20 insufflations of benzol produced no arhythmia, slight or no vascular changes and respiration was always arrested or greatly inhibited.

A partial transection of the spinal cord that is of special interest occurred in rabbit 765 (fig. 3 E). This section was made 24 hours before the arhythmia tests at the 6 C. The transection includes all of the lateral column on the right side and all of the left lateral column except a small area of the ground bundle between the dorsal and the ventral horns and a small area lateral to the dorsal horn. During the arhythmia tests blood pressure maintained a level of 105 to 85 mm. A bigeminal pulse followed the 5th, 6th and 7th insufflations. All three arhythmias were slow in appearing as if delayed by the lesion.

Remarks. It is apparent from the above partial transection experiments of the spinal cord at the 6 C that the "arhythmia impulse" is not blocked or in any way affected by transecting the dorsal and ventral columns, the so-called latero-ventral columns and the outer half of the lateral columns. When, however, a median section is made wide enough to include the inner half of both lateral columns or the lateral columns are completely divided, the bigeminal arhythmia from benzol insufflation is completely blocked. The conclusion is then reached that the inner half of the lateral columns contains the arhythmia conducting fibers and one experiment indicates that the important part of this area is situated between the dorsal and the ventral horns.

Discussion. In the first paper of this series it was shown that it required several inhalations or one or more insufflations (20 in one animal) of an irritating vapor to bring on a bigeminal arhythmia. Also this arhythmia never appeared simultaneously with the onset of the stimulation as was the case with the arrest of respiration, rise in blood pressure and slowing and strengthening of the pulse. Since these accompanying phenomena are not the direct cause of the arhythmia, this suggests the possibility that the bigeminal arhythmia following benzol insufflations and the other methods of stimulation is not a direct reflex from the peripheral end organ to the heart, but rather that some part of the brain, presumably some portion of the formatio reticularis of the brain stem, may be stimulated very strongly or by a series of stimulations until it gives rise to an impulse or series of impulses, which descend the spinal cord and reach the left ventricle by way of the sympathetics. The region of the spinal cord traversed by these impulses contains the lateral reticulo-spinal (bulbospinal) fibers, which take origin from the formatio reticularis of the brain

stem. The importance of these nuclei was noted in earlier papers (1927a, b), where it was shown that the formatio reticularis cells probably synapsed with the sensory root fibers from many of the cranial nerves, with efferent fibers from the cerebral cortex, superior colliculi and the cerebellum. In paper b it was shown that the ground bundle fibers of the spinal cord were important for conducting impulses from the cerebral cortex and the superior colliculi that affected respiration.

On the other hand the delay of the bigeminal arhythmia elicited from insufflations and other means may be due to peripheral rather than central causes. In some animals the heart may require but one or more of these sympathetic stimulations to produce these premature ventricular contractions while others demand a bombardment or several bombardments. It is possible, though it seems unlikely from several of the experiments of the second paper of this series, that the nutrient supply to the heart is affected sufficiently by these sympathetic stimulations to produce the arhythmia. That this arhythmia is due to a spasm produced from constriction of the coronary vessels is not supported by the following statements: 1. It appears settled for some animals that the constrictor fibers for the coronary arteries run in the vagus and the dilators traverse the sympathetics, the system which evokes the arhythmia. 2. Anrep and Segall have shown a rise of the aortic blood pressure to be one of the chief factors for producing an increased coronary output and our arhythmia has invariably been preceded by a pronounced rise of the arterial pressure.

It is possible that this "arhythmia impulse" which has been blocked through severance of the first to the third thoracic spinal roots or by removal of the stellate ganglia may later be taken over by certain more caudal cardiac nerves arising from the 3–6 thoracic sympathetic ganglia. Such nerves have been described by Mivart for the cat, Perman for the calf, Cannon, Lewis and Britton for the cat, Kuntz for man, and Ionescu and Enachescu for the cat, dog, calf, sheep and man. No experiments, however, have been carried out to determine whether these cardiac nerves may later acquire the function of conducting this impulse.

SUMMARY AND CONCLUSIONS

Sectioning the vagi, depressors, cervical sympathetics, phrenics and removal of the inferior cervical sympathetic ganglia in one animal is favorable for an early appearance and for a prolongation of two separate intervals of the bigeminal arhythmia elicited from insufflations, mechanical or faradic stimulations of the nasal septum or from faradic stimulations of the central end of the vagus nerve.

A transection of the spinal cord at the level of the sixth or seventh cervical vertebra absolutely blocks a bigeminal pulse from insufflations.

Sectioning the spinal cord from the third to the sixth thoracic vertebrae

permits a bigeminal arhythmia from insufflations in some rabbits and in others it prevents it. Transecting the spinal cord from the sixth to the ninth thoracic vertebrae allows an arhythmia from insufflations, but usually it delays it.

In no instance has a bigeminal arhythmia been obtained from insufflations or other means used after both stellate (first thoracic sympathetic) ganglia were removed.

A bigeminal pulse has been readily obtained from benzol insufflation from every rabbit in which the left stellate had been removed and in every rabbit in which the left stellate had been removed and the right sympathetic cord had been cut directly below the stellate.

About two-thirds of the rabbits which had the right stellate extirpated or the right stellate extirpated and the left sympathetic cord cut below the stellate produced a bigeminal pulse readily from insufflations and about one-third gave no arhythmia.

Sectioning the last cervical and the first and second thoracic spinal roots in two rabbits and the first three thoracic spinal roots in one rabbit prevented a bigeminal pulse from benzol insufflation.

A bigeminal arhythmia elicited from insufflation and other means is blocked by transecting the following areas of the spinal cord at the sixth cervical vertebra: a, both lateral columns: b, the dorsal and ventral columns and the median half of the lateral columns: c, the lateral and dorsal columns.

This arhythmia is not blocked at the 6 C from transecting: a, the dorsal and ventral columns (a few of the outer fibers in one of the "latero-ventral columns" were intact); b, the right lateral column and the outer half of the left lateral column in one animal.

It is concluded from these experiments that the bigeminal arhythmia produced in rabbits from insufflation and other means is caused by a nervous impulse or impulses which descend the median half of the lateral columns of the spinal cord and pass to the left ventricle by way of the upper thoracic roots and the stellate ganglia. This is obviously an action not usually attributed to the sympathetics.

The writer desires at this time to express his obligations to Dr. A. H. Schwichtenberg who assisted in the early experiments of this problem and to Mr. L. S. Goodman who assisted in all but the first experiments.

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NOTE. After the manuscript for this paper was practically completed there appeared in the September 6 number of the Journal of the American Medical Association a preliminary paper by Brow, Long and Beattie on the "Irregularities of the Heart under Chloroform." These irregularities which consisted of extrasystoles and tachycardia were said to disappear when the stellate ganglia were removed and when a portion of the brain situated between the caudal border of the optic chiasma and the caudal border of the mamillary bodies and the cephalic border of the superior colliculi was not intact. It is of interest to note that this arhythmia which seems to be associated with a damaged heart depends on the cardiac sympathetics for its continuance.

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